JOURNAL OF DAIRY SCIENCE

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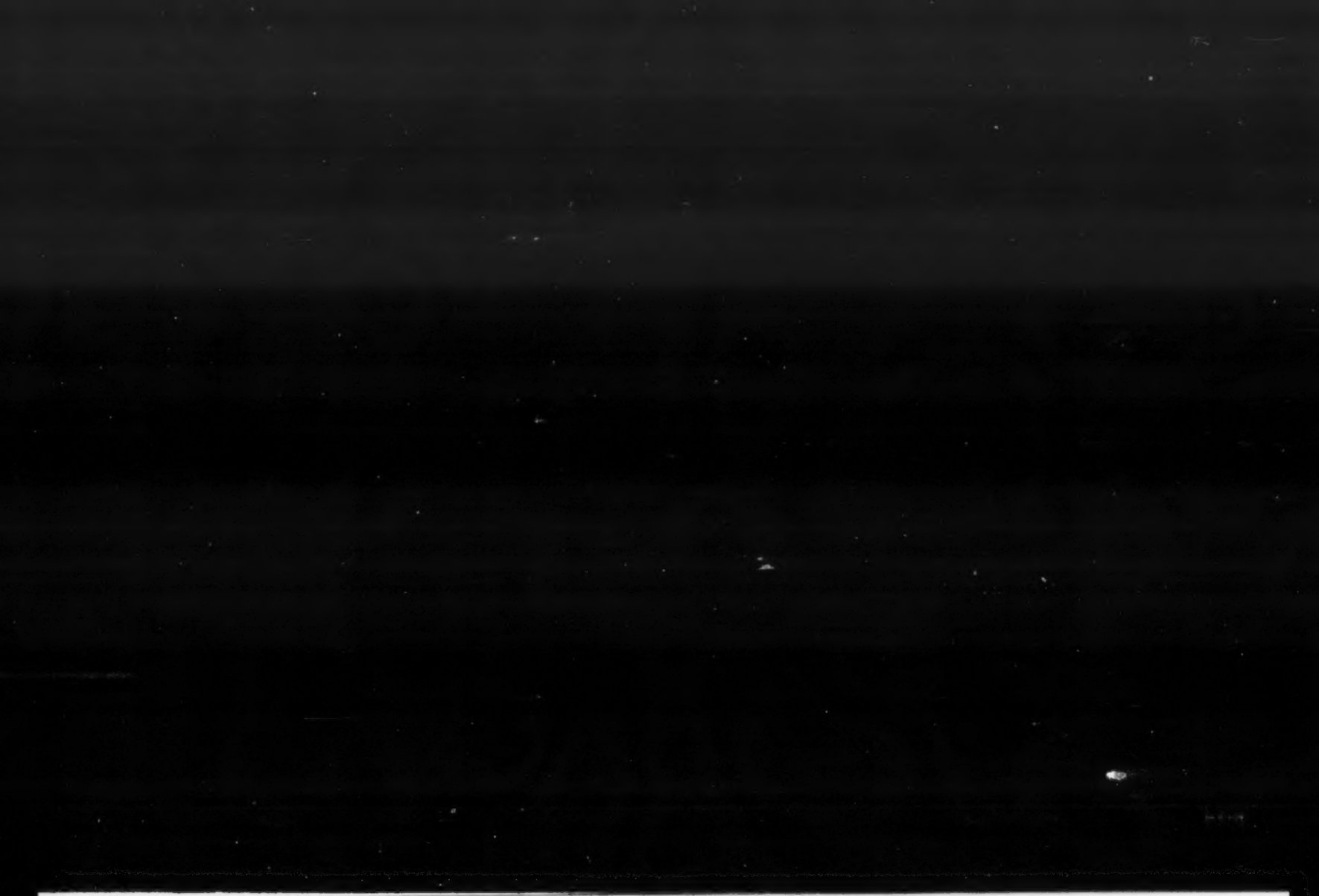

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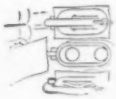

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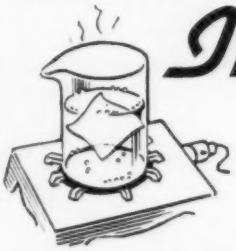

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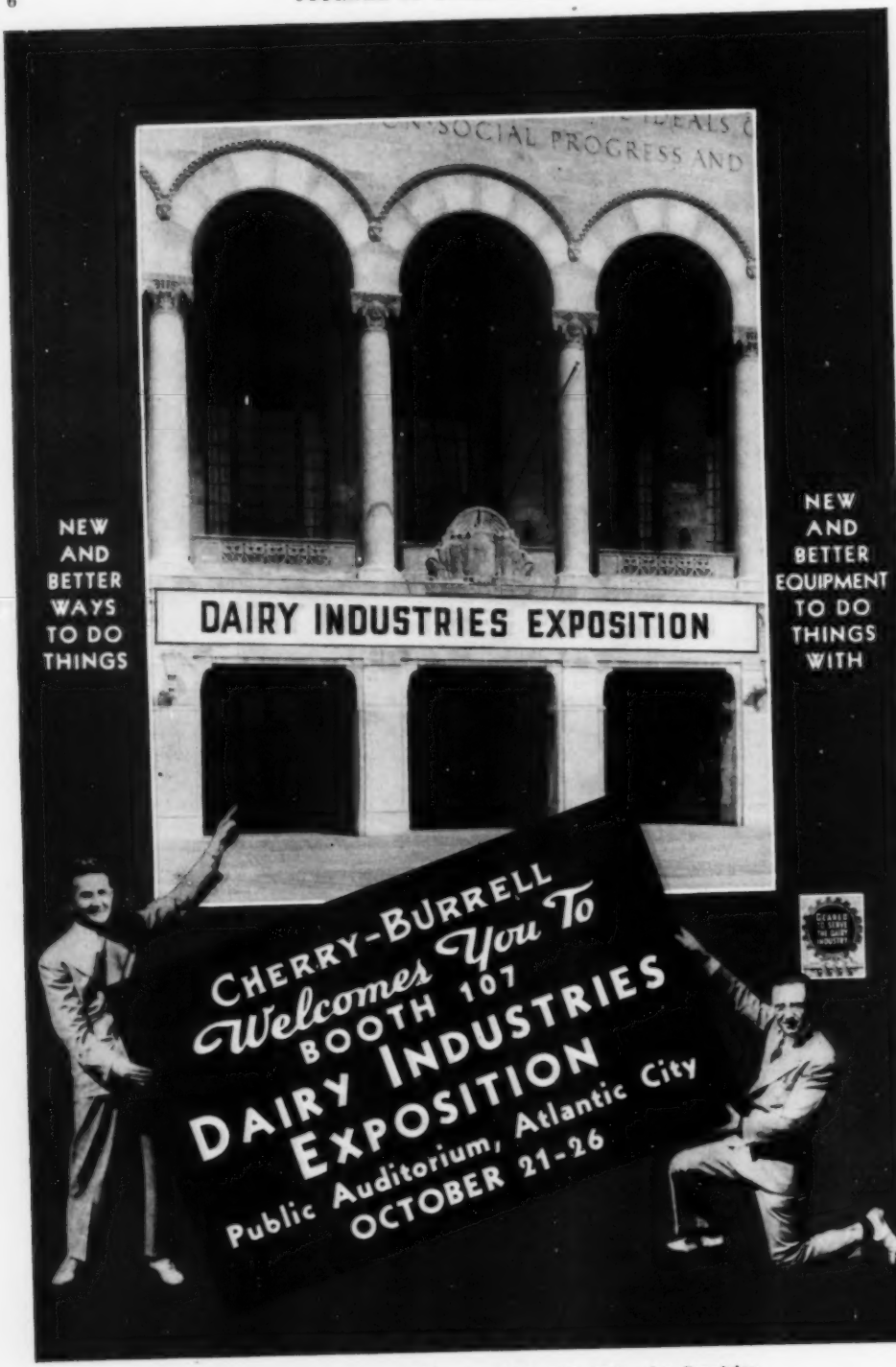

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIII OCTOBER, 1940 NUMBER 10

THE INTENSITY AND KIND OF SELECTION ACTUALLY PRACTICED IN DAIRY HERDS*

DWIGHT M. SEATH[1]

Iowa Agricultural Experiment Station

It is important to know how much selection among females actually occurs in dairy herds. Knowing what causes certain cows to remain in herds while others leave, is also important if we are to learn how to make more rapid improvement in dairy cattle through selection.

Attempts to measure the yearly replacements necessary in a herd and thus arrive at the average productive life of dairy cows have been numerous. Spillman *et al.* (19) used this method and estimated the productive life at 4.34 years for cows in Pennsylvania dairy herds and at 4.52 years in Michigan herds. McCandlish (11) gave 3.5 to 4 years as the productive life of dairy cows and Alexander (1) found that the Iowa State College herd had averaged 3.59 years. A report by Smith *et al.* (17) however, showed a shorter productive life (3.17 years) in the milking herds of West Sussex. Lush and Lacy (10) from a study of 500 cows in each of the dairy breeds found the productive life 3.5 years.

A study concerned with the increase in average production of a herd was reported by Gooch (5). She concluded from her study of 1741 eight-month lactation records over a period of 18 years that part of "this (increase) may have been brought about by selecting cows with comparatively high initial yields, since such cows have a high eight-months' total." A study by Plum (13) in Denmark of the Kollekolle herd for the years 1900 to 1934 revealed an 80-kilogram increase in butterfat production of which he attributes 10 kilograms (22 pounds) to the selection of females. This showed an average yearly improvement of .65 pounds of butterfat.

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* Journal Paper No. J-728 of the Iowa Agricultural Experiment Station, Ames, Iowa.

[1] Now with Louisiana Agricultural Experiment Station, University, Louisiana. The author is indebted to Dr. J. L. Lush, Dr. C. Y. Cannon and Dr. J. W. Gowen of Iowa Agricultural Experiment Station, for their many suggestions and criticisms during the conduct of this study. Acknowledgment is also given to the Kansas Agricultural Experiment Station, and to the Agricultural Extension Services of Iowa State College and of Kansas State College for their help.

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MATERIAL AND METHODS

The present study was conducted in order to measure the influence that culling has on the average production of cow testing association herds. "Culls" as defined in this study included all cows which finish a cow testing association year but leave the herd before the completion of the following association year. The "culls" thus included cows leaving herds for all reasons, *i.e.*, those that died, as well as those sold for dairy purposes, for beef, or because of disease, et cetera.

Iowa and Kansas Cow Testing Association data were used in this study. In Iowa, records on 147 herds, representing three herds taken at random from each of 49 Iowa Cow Testing Associations, were used. Records on 37 Kansas herds were also studied. Each herd had been tested continuously from 3 to 6 years in its respective state during this period. The Kansas herds were those having the most complete records for the period studied.

The yearly cow testing association milk and butterfat records were used as a measure of a cow's productivity. After her first calving each cow was considered as "on test" as long as she remained in the herd. There was, of course, variation in whether the cow was actually in milk for the entire 12 months of the period or for only a part of the time, but no corrections for this were made. The age-corrected yearly association records were considered reliable enough for this investigation, inasmuch as they have been shown (7) to repeat themselves in succeeding years about as closely as do age-corrected lactation records.

A total of 4495 different cows were included in the 147 Iowa herds studied. These cows had a total of 8010 records. Within the 37 Kansas herds there were 1883 different cows which had a total of 4087 records.

Inasmuch as the cow's age in most cases was given in even years, it was necessary to use a single age-conversion factor for all animals of a given year of age. Based on findings (12) about freshening ages in Iowa Cow Testing Association herds, the two-year-olds were assumed to be two years and two months of age. Each age group thereafter was likewise considered as the even year plus two months, leaving twelve months as the interval between groups. Bureau of Dairy Industry (9) age conversion factors for these ages were used.

Incomplete records of cows were handled in two ways. If a cow had a previous record and was not in milk at least 10 months during the association year, the incomplete record was not used and her productive ability was based on the previously completed record. Obviously most incomplete records of cows three years and over fell into this group. Two-year-old and a few three-year-old incomplete records were treated differently. For these it was necessary to raise the records to a complete mature-equivalent basis. If this had not been done, any tendency for herd owners to base their culling of heifers on the early production of a first lactation would not have been

found in this investigation, for such heifers have no previous records to indicate their productive abilities.

Conversion factors for raising records to a complete mature basis were used on all records of first-calf cows on test less than 11 months, whether made by animals leaving the herd or by ones remaining. The fact that adjustments were made on those remaining in the herd, as well as on those leaving the herd, would tend to balance any error arising from imperfections of these adjustment factors. These adjustment factors were computed from the Iowa data by choosing at random among cows of a similar age those which had incomplete records their first year but were on test for 12 months the following year.

RESULTS OF STUDY

Year to year variations

Animals leaving herds for all reasons and thus falling into the "cull" classification constituted 28.6 per cent of Iowa herds and 30.9 per cent of Kansas herds. This turnover indicated a productive life of 3.50 years in Iowa Cow Testing Association herds and 3.24 years in Kansas Association herds. These averages would be slightly higher if allowances were made for cows bought after having spent part of their productive lives in other herds, and for cows sold into other herds where they would yet spend a part of their productive lives.

TABLE 1

Total cows and culls with percentage of culls by years

Year	Iowa			Kansas		
	Total Cows	Number Culls	Per cent Culls	Total Cows	Number Culls	Per cent Culls
1930	.....	.....	.....	289	85	29.4
1931	1665	433	26.0	603	195	32.3
1932	2100	537	25.6	778	234	30.1
1933	2009	585	29.1	804	237	29.5
1934	1600	518	32.4	838	309	36.9
1935	636	215	33.8	775	204	26.3
Total or average	8010	2288	28.6	4087	1264	30.9
	$X^2 = 34.8$, $P < .001$			$X^2 = 24.4$, $P < .001$		

Highly significant differences[2] were found between the amount of culling by years (table 1). The lowest percentage of culls during the years studied was for Iowa in 1932 when 25.6 per cent of the cows were culls, while the highest percentage of culling occurred in Kansas herds during the drouth year of 1934 when 36.9 per cent of the cows became culls. Likewise, the drouth caused heavy culling in Iowa herds with 32.4 per cent culled in 1934 and a still higher percentage (33.8 per cent) leaving herds in 1935. Differ-

[2] Throughout this study, probabilities of .01 or less were considered as highly significant while significant probabilities were taken as those lying between .05 and .01.

ences in assigning individual cow testing association records to a given year are thought to account for these two years of heavy culling in Iowa herds as contrasted to the one year (1934) in Kansas.

TABLE 2

Averages and differences between production of culls and non-culls

	All cows		Non-culls		Culls		Difference: non-culls minus culls		Difference: all cows minus non-culls*	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
Iowa	8985	359	9355	374	8061	320	1294	54	370	15
Kansas	8379	348	8633	362	7812	316	821	46	254	14

* Selection differential.

Culls on the average produced less milk and butterfat for each of the years studied than did the non-culls. In Iowa herds the average non-cull excelled the average cull (table 2) by 1294 pounds of milk and 54 pounds of butterfat annually. Obviously there was no clear-cut division between the productive levels of the two groups (see figure 1), for among the culls were some good producers and among the non-culls were some poor producers. Kansas non-culls produced an average of 821 pounds of milk and 46 pounds of butterfat more than the culls. Variation as between years ranged from a difference in milk production of 752 pounds (Kansas, 1933) to 1837 pounds (Iowa, 1931). Butterfat differences varied from a low of 37 pounds (Kansas, 1934) to a high of 73 pounds (Iowa, 1931). In both states the heavy culling attributable to the 1934 drouth reduced the production spread between the two groups.

The spread between the butterfat production of culls and non-culls when tested for significance by the analysis of variance (18) showed highly significant differences for each of the years studied within each state. The portion of the total variance attributed to differences between the two groups varied from 3.3 to 10.1 per cent for Iowa and from 2.7 to 5.8 per cent for Kansas.

The selection differential which is computed by determining the difference between the production of non-culls and all cows (table 2), shows how much higher the herd average would have been in that year if the culls had been removed before the year began. For the five years in Iowa this selection differential averaged 370 pounds of milk and 15 pounds of butterfat, and in Kansas the six year average was 254 pounds of milk and 14 pounds of butterfat.

Herd differences in culling

Differences in herd practices and feed available, as well as the owner's financial needs, are among the factors which, in addition to differences in the

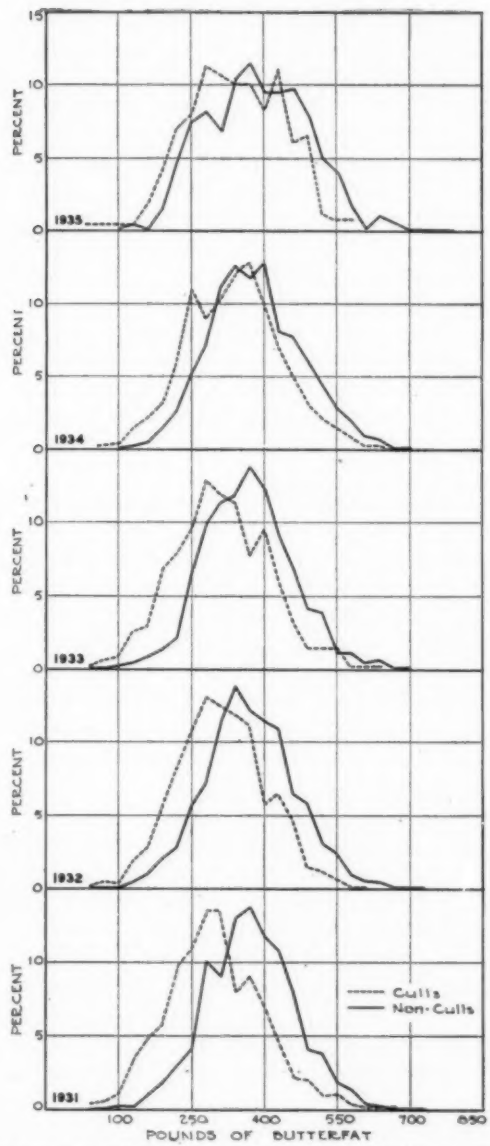

FIG. 1. Iowa non-culls and culls grouped according to butterfat production level.

percentages of low-producing and diseased cows, could cause variations in the amount and intensity of culling between herds. Actual differences found in percentage culled between the 37 Kansas herds varied from a low of 17 per cent to a high of 49 per cent. The herd differences in percentage culled were highly significant and gave evidence that factors affecting the herd as a whole caused some herds to have a high percentage of culls and other herds to have a low percentage of culls.

In general, there was evidence that Kansas herds having a high percentage of culls also had a wide spread between the production of non-culls and culls. A few striking exceptions to this trend were found, however, and no effort was made to measure its statistical relationship.

The selection differentials by herds also showed a wide range in Kansas. In one case the entire herd actually averaged 66 pounds more milk than the non-culls. The most evidence of culling for milk production was for a herd whose non-culls produced 740 pounds more milk on an average than all the cows. The range in butterfat selection differential was from zero to 33 pounds.

The method of fitting constants (21) was used in the Iowa study to remove the effects of disproportionate frequency and thus to permit testing the data for "interaction" between herds and differences in the butterfat production of non-culls and culls. A real interaction was found for each of the five years studied. This means that among the 147 herds some were culled for production much more severely than others.

Inasmuch as positive evidence was found in the Iowa and Kansas studies that the amount as well as the intensity of culling for production varied from herd to herd, this phase of the investigation was pushed further with Iowa data in an effort to learn whether these characteristics peculiar to a herd continued year after year.

The first phase of this investigation concerned the selection differential, *i.e.*, the amount that non-culls excelled all cows. Low positive correlations existed between the selection differentials of a herd in consecutive years, ranging from .02 to .13 with an average of .09.[3] They were not statistically significant. For non-consecutive years the correlations varied from .11 to .34 and averaged .23, which was highly significant. Why non-consecutive years showed a higher degree of association than consecutive years is not readily apparent. One plausible explanation is that a man might severely cull his herd for production one year but, because of a lack of replacements the next year, be obliged to wait until the third or fourth year to duplicate the process.

As might be expected, close relationships were found between the percentage culled and the selection differential. The correlation ranged from .11 to .56 and averaged .36.

[3] All correlation averages were computed by the Z method, Fisher, (4).

The herd's average butterfat production did not, however, reveal any such consistent relationship to the selection differential and had two negative correlations (−.03 and −.02) and two positive correlations (.18 and .09), averaging .06 for the four years studied. These results would indicate that the herds with high herd averages were able to practice on an average as intense, if not slightly more intense selection for high production as did herds with lower butterfat averages.

Low, but non-significant, relationships were found between the size of herd and the selection differential, indicating that as the size of the herd increased the selection differential tended to be slightly larger. The correlations by years varied from .02 to .47 and averaged .13. *A priori* it would not seem that size of herd would have this effect, since the percentage of replacements would be about the same in large and in small herds. There might, however, be management practices associated with herd size which would help or hinder culling in herds of some sizes more than in herds of other sizes.

Correlations between consecutive years for percentage of herd culled gave further evidence as to the degree to which voluntary selection was a characteristic of herd management. Low correlations averaging .10 and ranging from zero to .15 were found. Percentage of animals culled was obviously not entirely the result of voluntary culling by men in charge of herds but includes also a great deal of irregular and involuntary culling governed partially by the number of replacement animals available.

Breed differences in culling

A comparison of the percentage of purebreds, grades and scrubs culled in Iowa and Kansas Association herds showed (table 3) one striking difference as between states. In Iowa the percentage culled among purebreds (28.2 per cent) was not significantly different from the percentage culled (28.7 per cent) among the grades. Kansas, on the other hand, with 26.7 per cent culls among purebreds and 35.4 per cent culls among grades, displayed almost one-third more culling among grades than among purebred animals. The high percentage of culling of Kansas grades could partially have been caused by the presence of a number of herds that had both grades and purebreds. The owners of these herds may have been eager to convert their herds rapidly to wholly purebred ones and, therefore, may have done most of their voluntary culling of grades. The 80 scrubs in Iowa herds were culled more heavily (table 3) than either the purebreds or grades.

The intensity of culling as measured by the production spread between the non-culls and culls was greater in each state for purebreds than for grades (table 3). In Iowa the purebred non-culls excelled the purebred culls by 2696 pounds of milk and 57 pounds of butterfat, whereas the grade non-culls produced 1321 pounds of milk and 53 pounds of butterfat more

TABLE 3

Intensity and homogeneity of culling among purebreds, grades and scrubs

	Iowa				Kansas			
	Cows		Production difference of non-culls and culls		Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat
Purebred	2918	28.2	2696	57	2124	26.7	776	49
Grades	5011	28.7	1321	53	1953	35.4	708	41
Scrubs	80	31.2	1814	62	*			

* Three scrubs, 3 Brown Swiss and 4 Red Polled cows were eliminated from the Kansas study of breeds because there were so few in each class.

than the grade culls. Kansas data revealed this same trend with the purebreds excelling in both comparisons between non-culls and culls with a milk yield difference of 776 pounds for purebreds and 708 pounds for grades, and a butterfat yield difference of 49 pounds for purebreds and of 41 pounds for grades.

Between-breed differences in amount of culling were found in both the Iowa and Kansas studies, yet the results were not consistent as between states. Iowa herds culled a strikingly large percentage of grade Guernseys (32.6 per cent) and a relatively small percentage of purebred Guernseys (18.1 per cent), while in Kansas purebred Holsteins and Grade Ayrshires with 29.5 per cent and 49.0 per cent culls ranked first, respectively, in culling among the purebred and grade groups. The least amount of culling among Kansas breeds was for purebred Jerseys with 22.4 per cent culls. Chi-square values derived from tests for homogeneity of culling between breeds gave significant figures for each state. These values would likely have been less had the influence caused by the general differences between herds been first removed, for it has already been shown that factors affecting the herd as a unit had an influence on the amount of culling done. Inasmuch as most herds consisted largely of one breed, the heavy culling of a herd would exert its culling influence largely on one breed.

Differences between the production of non-culls and culls by breeds were irregular as between states, thus failing to indicate that one breed selected more closely for either high milk or high butterfat production than did the others.

How months in milk affected culling

The production records used in this study were for a cow testing association year rather than for a lactation. The period that the cows were in milk showed (to the nearest month) the time during an association year that the

cow was in production. The months stated thus represented the part or parts of one or of two lactations that a cow was milked.

When the cows were grouped by months in milk, differences in the percentage culled during the next year were significant in both states (top half of table 4). Before making these comparisons the two- and three-year-olds with incomplete records were removed from both the cull and non-cull groups, so that no discrepancy would be introduced by their inclusion. A distinct tendency was found to cull heavily the cows that were in milk only a small part of the preceding year and to retain a higher than average percentage of those in milk ten or more months. Forty-eight per cent of the Iowa cows and 40.9 per cent of the Kansas cows in milk five or less months were culled. This was in contrast to an average culling among these groups of 26.5 per cent in Iowa and 29.0 per cent in Kansas. Cows in milk eleven months experienced the least culling, 24.3 per cent in Iowa and 26.8 per cent in Kansas.

The milk and butterfat production differences between non-culls and culls (top half of table 4) showed no uniform trend either within states or between states that could be ascribable to months in milk. For certain years, however, there was evidence that a definite interaction did exist between months in milk and the butterfat production spread between culls and non-culls. While not tested in the Kansas study, evidence of such interaction was found in Iowa for 1931, 1932, and 1933. The years 1934 and 1935, however, showed a definite lack of interaction. The lack of relationship in 1934 and 1935 may have been due to the influence of the drouth which could have upset normal culling practices.

The culling among two- and three-year-olds (bottom half of table 4) having incomplete first lactations averaged 35.0 per cent in Iowa and 37.1 per cent in Kansas, and was significantly heavier than the 26.5 per cent and 29.0 per cent respectively shown in these states for other cows (top half of table 4). The culling of these young cows was comparatively heavy for each of the months-in-milk groups with no apparent uniform trend as between states.

The production spread between culls and non-culls, as with the percentage of culling, was also greater for these two- and three-year-olds with incomplete records (raised to a full-time basis) than for other cows—in Iowa 2017 pounds of milk and 74 pounds of butterfat as compared to 559 pounds of milk and 40 pounds of butterfat for other cows, and in Kansas 970 pounds of milk and 56 pounds of butterfat as compared to 717 pounds of milk and 41 pounds of butterfat for other cows. This evidence of early selection based on the first production records of cows is further supported by the larger production spread between the culls and non-culls during the early part of these first lactations (bottom half of table 4). Within each state this spread was the greatest for one month in milk, with those in milk two

TABLE 4

The amount and intensity of culling as influenced by months in milk during preceding year

Mos. in milk	Iowa				Kansas			
	Cows		Production difference of non-culls and culls		Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat
Complete records								
2	3	48.0	4450	245	3	40.9	1400	12
3	4		1567	60	3		−1350	−60
4	6 (25)		−1860	−46	4 (22)		−1500	−60
5	12		−2613	10	12		−1213	−21
6	26	46.2	830	33	24	41.7	−730	−28
7	66	39.4	657	35	38	47.4	596	19
8	304	35.2	857	38	167	35.9	1155	52
9	733	28.8	780	30	406	31.3	586	29
10	2361	25.8	1048	45	1136	28.3	512	31
11	1669	24.3	567	33	889	26.8	922	51
12	839	25.4	706	37	437	27.7	5	15
Total or average	6023	26.5	559	40	3119	29.0	717	41
	$X^2 = 35.9$, $P < .001$				$X^2 = 17.3$, $P < .01$			
Incomplete 2- and 3-year-olds—records raised to full-time basis								
1	137	38.7	3174	119	108	35.2	1754	108
2	239	33.5	2360	84	126	26.6	956	58
3	231 (1293)	32.0 (35.9)	1719	77	118 (642)	30.5 (35.2)	1288	66
4	244	34.8	2403	84	86	36.0	1499	36
5	199	38.2	2642	96	116	45.7	866	47
6	243	39.5	2008	73	88	36.4	−695	7
7	181	34.3	1936	78	68	33.8	403	47
8	223	38.6	1639	50	72	38.9	413	40
9	163	31.3	600	28	90	50.0	825	48
10	123	24.4	741	18	96	38.5	1647	82
Total or average	1983	35.0	2017	74	968	37.1	970	56

and three months also showing a wide production spread between the two groups.

These findings warrant the conclusion that much early culling based on production takes place during the first lactation of two- and three-year-olds in both Iowa and Kansas Cow Testing Association herds.

Age and its influence on culling

The influence of age on the number culled and on the production spread between culls and non-culls was studied by using the ages of the cows given (in whole years) for the year prior to culling (the incomplete record two- and three-year-olds excepted). This placed a large portion of the cows in an age group one year less than the actual age when they left the herd.

Both Iowa and Kansas herds showed a definite effect of age of cows on culling rates. (See figure 2 for distribution by ages of Iowa culls and non-culls.) In Iowa the culling rates among cows less than eight years old were

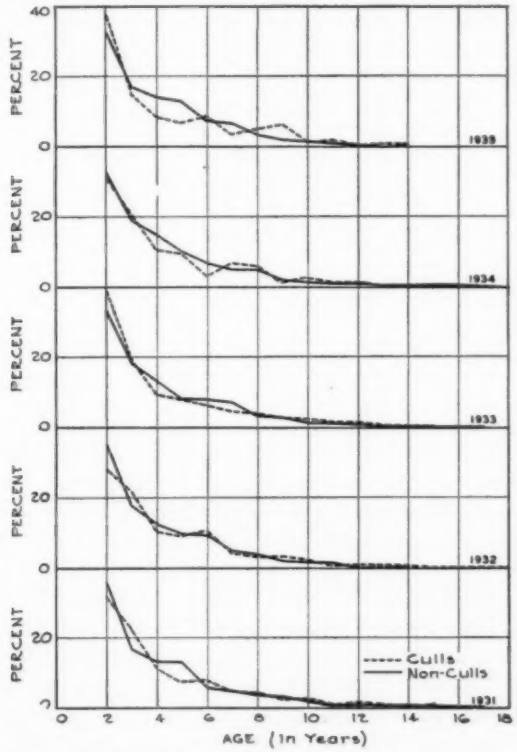

FIG. 2. Distribution of culls and non-culls by ages (Iowa).

the heaviest for three-year-olds with 31.3 per cent (table 5) of their number culled. The two-year-olds were second with 28.1 per cent culled. Kansas herds culled the two-year-olds most heavily (31.6 per cent) and the three-year-olds second (30.4 per cent). An important part of this heavy culling of two- and three-year-olds as previously shown was among those with incomplete first-lactation records.

TABLE 5

The percentage of culls by ages (Ages given for year prior to culling)

Age (years)	Iowa				Kansas			
	Cows		Production difference of non-culls and culls		Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat
2	2705	28.1	1659	63	1325	31.6	1107	57
3	1500	31.3	1764	75	723	30.4	1102	54
4	995	23.2	649	34	489	27.2	766	53
5	800	25.0	647	38	403	26.8	549	37
6	616	27.4	1200	47	337	26.1	598	27
7	448	26.3	993	46	274	29.6	192	27
8	337	31.8	838	32	216	32.9	321	41
9	231	34.2	744	40	135	42.2	−166	35
10	164	38.4	1367	52	72	44.4	1334	36
11	94	33.0	1422	60	46	45.7	569	20
12	60				26			
13	31				18			
14	15				11			
15	8	50.7	197	12	7	50.7	1682	54
16	2				4			
17	2				1			
18	2							
Total or average	8010	28.6	1294	54	4087	30.9	821	46
X^2		69.2				47.4		
d/f		10				10		
P		< .001				< .001		

Advancing age caused a decided increase in culling as evidenced by the heavy culling of cows eight or more years of age (table 5). Especially heavy culling took place among cows twelve years or older, with an average of 50.7 per cent of those leaving the association herds in both Iowa and Kansas.

The production differences between the non-culls and culls by age groups (table 5) were large for both milk and butterfat production for the heavily culled two- and three-year-old groups. In Iowa the three-year-olds were the most intensely culled, their non-culls excelling their culls by 1764 pounds of milk and 75 pounds of butterfat. In Kansas the two-year-old non-culls had the largest spread over culls of any of the age groups with 1107 pounds of milk and 57 pounds of butterfat. In Iowa the two-year-olds ranked sec-

ond, while in Kansas the two classes were reversed with the three-year-olds second. Irregular production differences having no particular trend were found for groups 12 years old or over.

The influence of body weight

Weight was studied as a possible factor which might have influenced selection, inasmuch as various studies have shown that larger cows generally produce more milk and butterfat than do smaller ones.

The study of the influence of weight on culling was confined to Iowa data where the estimated weights on the yearly reports of the testing supervisors were used. Such data were available on approximately 80 per cent of the cows within the 147 herds. Although these weights were only estimates of the herd owner and testing supervisor, and of course would vary from year to year, they did furnish some information on the general size of the animals.

Indications were found (table 6) that cows weighing 600 pounds or less were culled heavily, for 40 per cent of the cows falling into this group were culled as compared to an average of 28.8 per cent for the 7436 cows. Also, the cows weighing 1500 pounds or more showed a high percentage culled, with 35.3 per cent of them leaving the herds.

TABLE 6

Percentage culled and production differences between culls and non-culls as influenced by body weight (Iowa herds only)

Body weight (pounds)	Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat
600 or less	130	40.0	1041	58
700	289	30.8	746	42
800	1046	28.0	991	47
900	1031	27.4	985	47
1000	2048	28.0	1421	58
1100	1103	27.9	1675	65
1200	1026	31.0	1389	55
1300	365	29.6	1754	69
1400	282	25.9	1273	57
1500 and over	116	35.3	69	9
Total or Average	7436	28.8	1282	54

$X^2 = 15.4$, P .08

The significance of the differences found in number culled by weight groups when studied by chi-square for individual years left much doubt as a definite relationship of culling to body weight. In only one year (1933) was the probability less than .05 that culling intensity was independent of weight. When all data were combined for the five years the probability was .08. Only the extreme classes (600 pounds or less and 1500 pounds and

over) seem likely to have been culled with an intensity really different from that in other weight classes.

Differences between the production of non-culls and culls by weight groups (table 6) displayed little evidence of a trend. Only one year, 1931, had a significant interaction between weight and the difference in production. This was offset by the years 1932, 1933, and 1934 where the mean square due to interaction was actually less than that within the groups. Even though herd owners did cull more heavily the extremely light and extremely heavy cows, the production differences between the non-cull and cull groups within the various weight classes did not vary in a regular way with weight of the cow.

Why cows were culled

For the Kansas study herd owners furnished through their testing supervisor information as to why cows left the herd. In many cases the reason given had been recorded in the herd record book, thus representing the reason given at the time the animal became a cull. In instances where this notation had not been made, the owner relied on his memory for the reason for culling. In case the reason could not be recalled, it was classified in the questionnaire as "reason unknown." Fifty animals representing 4 per cent of the culls were thus classified.

Cows sold because of low production accounted for 9.4 per cent of total cows or 30.5 per cent of the 1264 culls (table 7), *i.e.*, 30.5 per cent equals 7.1

TABLE 7

Why Kansas cows were culled and production level of each group

Reason	Number culls	Per cent of		Av. milk	Av. fat
		Total culls	Total cows		
Bang's disease	168	13.3	4.1	7873	325
Udder trouble	133	10.5	3.3	8692	347
Sterility	92	7.3	2.2	7321	321
Died	83	6.6	2.0	8635	349
Misc. diseases	10	0.8	0.3	9100	413
Tuberculosis	8	0.6	0.2	7938	358
Total diseased	494	39.1	12.1		
Low producers sold for beef	296	23.4	7.2	7196	289
Low producers sold for dairy	90	7.1	2.2	6100	247
Good producers sold for dairy	240	19.0	5.9	8472	327
Old age	61	4.8	1.5	6328	341
Accidents	30	2.4	0.7	8360	334
Misc. reasons	3	0.2	0.1	7567	300
Reason unknown	50	4.0	1.2	7146	312
Grand total	1264	100.0	30.9	7812	316

per cent sold for dairy plus 23.4 per cent sold for beef. The low producers sold for dairy purposes had the lowest average production of any group—

6100 pounds of milk and 247 pounds of butterfat! The low producers sold for beef ranked second low in butterfat yield with 289 pounds of butterfat.

A total of 494, representing 39.1 per cent of the culls (12.1 per cent of total cows), left because of disease. Among the diseased groups those leaving because of sterility and Bang's disease produced the lowest yields of butterfat, 321 pounds and 325 pounds, respectively. Those afflicted with udder troubles averaged 347 pounds, just one pound less than the average of all cows.

Cows classed as good producers and sold for dairy purposes represented 19 per cent of the total culls. Their average production was 8472 pounds of milk and 327 pounds of butterfat. This was 93 pounds in excess of the average milk yield but 21 pounds less than the average butterfat yield of all cows. These dairymen apparently exercised some selection for higher butterfat percentage when animals were sold for dairy purposes.

The findings relative to percentage culled for various reasons (table 7) are about the same as the results reported (15) for a three-year period (1935–1937) on all Iowa and Kansas cow testing association herds, and are in close agreement with the Bureau of Dairy Industry's report (2) on a study made of 114,135 cows in 18 states. Culling for low production was strikingly similar between the three studies, ranging from 30.5 per cent (this study) to 35 per cent of the total culls. Losses due to disease in this study (12.1 per cent of total cows) coincided closely with the 13.5 per cent reported by Smith (17) in West Sussex cattle.

Age and the culling of low producers

As has been shown (table 7) 386 of the 1264 culls, or 30.5 per cent of all culls, were sold because of low production. When these 386 animals were classified by ages (table 8) a preponderance of them fell into the younger groups. Two-year-olds made up 40.2 per cent of their total, three-year-olds 23.8 per cent and four-year-olds 9.1 per cent, while only 4.4 per cent of them were over eight years of age.

TABLE 8

Ages and production level of cows sold because of low production

Age (years)	Number cows	Per cent of all culls	Average production	
			Milk	Fat
2	155	40.2	7055	278
3	92	23.8	6499	257
4	35	9.1	6540	269
5	26	6.7	7719	307
6	28	7.2	7278	311
7	14	3.6	7171	287
8	19	4.9	6647	265
9 and over	17	4.4	7541	292
Total or average	386	100.0	6942	277

The average mature-equivalent butterfat productive level of the various age groups (table 8) shows three-year-olds lowest with 257 pounds, while the two-year-olds averaged 278 pounds, and the four-year-olds 269 pounds. The 19 eight-year-olds were second low with 265 pounds while the highest average was registered by the 28 six-year-olds with 311 pounds. This group still produced five pounds less than the 316 pound average of all culls.

The indication that most of the culling for low production takes place early in a cow's productive life was confirmed in this portion of the study. Age relationships to culling, when all culls were considered, indicated this same trend (table 5).

When cows are culled

A sample of 414 culls having records (or possible records) through the two-, three-, and four-year-old periods were chosen in an effort to learn about the length of productive life and the number of freshenings of cows which are culled at early ages. Obviously many of these young cows did not have records for each of the three years studied, inasmuch as they became culls before they completed the three years in herds. In every case, however, care was taken to choose only records of cows that were in herds that were tested for three years following the beginning of these two-year-old records, so as to permit the securing of all their records, irrespective of whether they stayed in the herd for part or all of the three years.

The distribution of these culls for number of freshenings to time of culling showed that 50.7 per cent of the 414 had freshened but once, 32.8 per cent had freshened twice, 12.8 per cent freshened three times, and 3.6 per cent had four freshenings before being culled. The average was 1.7 freshenings to time of culling.

Of these 414 culls, 156 were cows that were classified by the owner as culled because of low production and sold either for beef or dairy purposes. For these low producers, the percentage distributions by number of freshenings were very nearly the same as those of the entire sample. Likewise, the average of 1.6 freshenings per cow is not very different from the average of 1.7 found for the entire 414 culls.

The average number of months elapsing from first freshening to time of culling for the 414 culls was 17.3 months. For the 156 culls that were sold because of low production it was 14.7 months, showing a greater difference than was apparent in the comparison between the two groups on number of freshenings.

The average period from last freshening to time of culling was 5.3 months for the entire group and 5.2 months for the low producers. This showed that the time of culling averages around the center of a cow's last lactation. The distribution of cows by months showed little tendency for the culls to have been sold mostly during specific months.

The effect of selection on inheritance of herds

If the selection differential represented only permanent differences between the cows, if these differences were 100 per cent hereditary, and if the choice of bulls represented culling of the same intensity, then it could be said that culling caused an annual improvement in the heredity of Iowa herds of 370 pounds of milk and 15 pounds of butterfat, and in Kansas of 254 pounds of milk and 14 pounds of butterfat (table 2). Unfortunately, the answer is not this simple. In the first place, the selection studied was only of cows and they contribute only one-half the genes to the next generation. Secondly, one record on a cow is only a partial indication of her real producing ability, and thirdly, only a fraction of the measured differences in cows is hereditary.

Now, if heredity were the only thing which caused records of the same cow to be alike, the intra-herd correlation between consecutive records of cows would represent the hereditary portion of the differences between single records of cows. In Iowa this correlation for butterfat yield was .38 for non-culls and .31 for culls and in Kansas .32 for non-culls and .27 for culls. These results are slightly lower than the .40 found by Plum (12) between consecutive lactation records in Iowa Cow Testing Associations. As was pointed out in that study, it is improbable that this 40 per cent (.40) of the intra-herd differences was entirely caused by differences between the heredity of cows, for there could exist intra-herd correlations between the environment to which the same cow had been exposed for different years. There might exist also a correlation between the inheritance of a cow and her environment, as when the cow with the best inheritance is given the best treatment.

The fact that culls had intra-herd correlations significantly lower than those for non-culls gives evidence that the butterfat differences actually found between the records of non-culls and culls for the year before the culls left the herd were somewhat larger than the real inherited differences. This fact plus the knowledge that all cows eventually become culls makes it logical to consider the intra-herd correlations between consecutive butterfat records of culls as the upper limit in heritability of butterfat yield, *i.e.*, .31 in Iowa and .27 in Kansas.

As a more dependable estimate of the importance of heredity in causing the observed intra-herd differences, dam-daughter correlations were computed on an intra-sire basis. The first record of each animal in dam-daughter pairs was used, so as to be affected as little as possible by selection. Resulting correlations before removing the effect of sire (including herd) were, in Iowa, .51 for milk and .28 for butterfat and, in Kansas, .57 for milk and .37 for butterfat. These results for milk are comparable to those of Gowen (6) who reported Jerseys at .30 and Holsteins at .50. On an intra-sire basis the correlations in the present study reduced for Iowa, to .10 for

milk and .07 for butterfat and, in Kansas, to .12 for milk and .02 for butterfat.

Doubling intra-sire dam-daughter correlations and adjusting for differences in the variance of daughters and dams provides an estimate of the importance of heredity in causing differences between records of cows kept in the same herd and mated to the same sire. This is identical with computing intra-sire regression of daughters' records on dams' records and doubling that in accordance with the principle of "diallel crossing" (14). Questions of whether the mating systems are random, inbreeding, or assortive mating based on somatic resemblance, are avoided by this method. This analysis yields only that fraction of the variance which can be expressed as due to additive gene interactions, as dominance deviations do not contribute to dam-daughter correlations and only a small part of the epistatic deviations do. In this narrow sense the fraction of the variance which is hereditary becomes:

Iowa: $2 \times .10 \sqrt{\frac{428.0}{376.8}} = .21$ (milk) $\quad 2 \times .07 \sqrt{\frac{65.8}{70.9}} = .13$ (fat)

Kansas: $2 \times .12 \sqrt{\frac{654}{580}} = .25$ (milk) $\quad 2 \times .02 \sqrt{\frac{108}{66}} = .05$ (fat)

Now if one adds to each of the above portions of the variance one-fourth of their respective values in line with Wright's (20) idea as the portion due to dominance or increases their value 50 per cent in line with Fisher's idea (3) in allowing for dominance, the importance of heredity (in this broader sense) increases. Wright's method would establish the lower limits in heritability which in this case would be, for milk, .26 (Iowa) and .30 (Kansas) and, for butterfat, .16 (Iowa) and .06 (Kansas).

Considering the intra-herd correlations between consecutive records of culls to be the upper limits of heritability for butterfat and the figures from the Fisher method as upper limits for milk (where the correlation between consecutive records was not studied), the range becomes:

Iowa:	.26 to .31 (milk)	.16 to .31 (fat)
Kansas:	.30 to .38 (milk)	.06 to .27 (fat)

Before extracting from the selection differentials the above portions attributed to heredity, it is necessary to correct the differential itself to allow for the low production of certain culls, *e.g.*, old and diseased cows during the last year they were in the herd. As evidence of this, among 1129 cows in Iowa herds all through 1933 and 1934, 368 became culls in 1935. During 1934 the non-culls averaged 14 pounds more butterfat than all cows but in 1933, a year earlier, this difference between the same cows was only 9 pounds.

To allow for this non-hereditary portion of the selection differential only two-thirds of the actual amount found was used in computing the hereditary portion, thus becoming, in Iowa, 247 pounds of milk and 10 pounds of but-

terfat and, in Kansas, 167 pounds of milk and 9.3 pounds of butterfat. Now if these characters were 100 per cent hereditary, only one-half of this would pass on to the next generation, inasmuch as each parent contributes equally to the offspring. Extracting this one-half from each (corrected) selection differential and applying the factors (cited above) representing the range in estimates of heritability, results in a net yearly hereditary improvement in milk yield of from 32 to 38 pounds in Iowa and from 25 to 32 pounds in Kansas. In butterfat yield the yearly improvement would be .80 to 1.55 pounds in Iowa and .28 to 1.25 in Kansas.

The indicated annual hereditary improvement in cow testing association herds thus resulting from the culling of cows as shown here would range (considering the results from both states) between 25 and 38 pounds of milk and .28 and 1.55 pounds of butterfat.

These culling benefits are not very different from the results reported by Plum (13) in his study of records covering 35 years in the Danish Kollekolle herd. He found an improvement of 80 kilograms of butterfat of which he stated that not over 10 kilograms was hereditary improvement brought about by the selection of females. This amount (22 pounds) would represent an average of .64 pounds per year, which falls between the limits of improvement found for butterfat in this study.

SUMMARY

In a study of 147 Iowa and 37 Kansas Cow Testing Association herds it was found that the annual exodus of cows averaged 28.6 per cent for five years in Iowa and 30.9 per cent for six years in Kansas. The 1934 drouth appreciably increased culling in both states.

For each year in both states the non-culls averaged higher in production than did the culls. The average production spread between the two groups was 1294 pounds of milk and 54 pounds of butterfat in Iowa and 821 pounds of milk and 46 pounds of butterfat in Kansas.

Herd differences were found both in percentage culled and in intensity of selection for high production.

Heavier than average culling was found among cows that only milked a few months the previous year. In Iowa herds 48 per cent of those cows that milked five or less months were culled, and in Kansas 40.9 per cent of this group were eliminated.

Heavy culling during the first lactation of two- and three-year-olds was found in each state with 35.0 per cent of such cows (having incomplete records) leaving Iowa herds and 37.1 per cent leaving Kansas herds. These culls showed appreciably lower production records than did other culls.

Ages of cows had an important relationship to culling. Two- and three-year-olds were heavily culled and showed an approximate 25 per cent increase in spread between the production of non-culls and culls as compared

with the average of all age groups. An average of 50.7 per cent of cows 12 years of age and over was culled in each state.

No consistent relationship was found between breeds and culling.

Iowa cows having estimated body weights of 600 pounds or less and 1200 pounds or more were heavily culled, yet too few cows fell into these two classes to permit definite conclusions to be drawn.

Reasons given for culling Kansas herds showed that 39.1 per cent of culls left because of disease. Only 6.6 per cent of the culls died. A total of 30.5 per cent of culls was sold because of low production and good producers sold for dairy purposes accounted for 19.0 per cent. Those sold because of sterility and Bang's disease showed the lowest previous years' production among the diseased cows. Among all groups the low producers sold for dairy purposes produced the least butterfat with low producers sold for beef next above them.

A study of 414 culls followed through the heavy culling ages, *i.e.*, two, three, and four years, revealed that the average number of freshenings to culling was 1.7; the months from first freshening to culling, 17.3; and months from last freshening to culling, 5.3.

The estimated yearly hereditary increase in herd average expected to result from the selection found in this study ranged from 25 to 38 pounds of milk and from .28 to 1.55 pounds of butterfat.

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RUMEN DIGESTION IN THE BOVINE WITH SOME OBSERVATIONS ON THE DIGESTIBILITY OF ALFALFA HAY[1]

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The importance of rumen digestion has been recognized for a long time but it has been only within recent years that dependable experimental evidence pertaining to rumen physiology has been obtained. Even now, little is known of the significance of rumen digestion when compared with the total digestion that occurs in the passage of feeds through the digestive tract. Recently, however, *in vivo* chemical studies of the digestion of various nutrients within the rumen have been undertaken. Silver (1) studied the digestion and absorption of alfalfa hay by removing the rumen contents at the time of feeding and at 2-hour intervals thereafter. He interpreted his results by comparing the various values for percentage composition of the rumen contents as the period of digestion progressed. Kick and Gerlaugh (2) studied the effect of the preparation of alfalfa hay on rumen digestion and reported the percentage of the total daily intake that was represented by the rumen ingesta when removed 24 hours after feeding. In both of these investigations it was found that protein disappeared rapidly from the rumen while fiber disappeared slowly. Quittek (3) and Krzywanek and Quittek (4) compared the percentage composition of the rumen contents removed at 3-hour intervals with that of the hay. They observed that the percentages of nitrogen, crude fiber and crude fat in the dry matter of the rumen increased as digestion progressed. The increase in these nutrients was attributed to the rapid disappearance of carbohydrates by rumen fermentation. Although all of these methods have served a purpose, they are inadequate in making studies of rumen digestion because the results can only be interpreted on a comparative basis and no idea of the quantitative digestion can be obtained.

In vitro studies of the products of cellulose fermentation by rumen and intestinal flora have been numerous. The recent studies of Woodman and Evans (5) and Pochon (6) demonstrated the formation of the lower fatty acids from the hydrolytic decomposition of cellulose and suggested that these acids may be the products of rumen digestion. Acetic and butyric acids were produced in considerable amounts while lactic, pyruvic, propionic, formic, and valeric acids and glucose were produced in smaller amounts. The findings of Trautmann (7) indicate that the rumen, reticulum and

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omasum are capable of rapid absorption of water and substances in solution. Davey (8) also obtained evidence of absorption from the stomach compartments of ruminants.

It has been generally assumed that the various nutrients of feed-stuffs are less digestible at the higher planes of nutrition. Watson and co-workers (9) have pointed out that the data supporting this are conflicting and indicate that some nutrients may be affected while others are not. Watson, Muir and Davidson (10) found that the level of feeding affected the utilization of rations of roughage alone differently than mixed rations.

The limitations of the present empirical determination of crude fiber were discussed by Crampton and Maynard (11) and they proposed the division of the carbohydrate fraction of feedstuffs into lignin, cellulose and other carbohydrates. Horwitt, Cowgill and Mendel (12) reviewed the long recognized faults of the ether extract method for the determination of fat and developed a relatively rapid method for the determination of true fat. They also investigated the value of an enzymatic method for the determination of crude fiber. There is a great need for an extension of the application of these and similar methods to feed analysis in order to determine their adequacy in the estimation of the feeding value of various roughages.

The present study was undertaken (a) to develop a method for evaluating quantitative digestion in the rumen, (b) to observe the effect of the plane of nutrition on digestion coefficients and rumen fill, and (c) to obtain information on the value of determining lignin, cellulose, crude fiber by an enzymatic method, and true fat in alfalfa hay.

METHODS

Six Holstein cows, five normal and one with rumen fistula, received alfalfa hay as the only feed in amounts of 10, 20 or 30 pounds per day. The rumen ingesta was removed from the cow with the fistula following a preliminary feeding period of at least 12 days at each level of feeding. The rumen contents were removed in the morning just before feeding (14 hours after the previous feeding) on six occasions (twice for each level of feeding), weighed, mixed, sampled for chemical analysis and replaced in the rumen. On one occasion the rumen contents were removed 24 hours after feeding rather than at the usual 14-hour period. The cow was weighed just before and after the rumen was emptied in order to check the weight of the material removed. A sample of feces was collected with each sample of rumen contents. Eleven digestion trials were conducted to supplement the results obtained with the rumen fistula cow. Three trials were at the 30-pound level, six at the 20-pound level and two at the 10-pound level. Each trial consisted of a 10-day preliminary period followed by 10 days of collection.

The chemical analyses which were made on the hay, rumen contents, and feces were moisture, protein, ash, ether extract, crude fiber, nitrogen-free extract and iron by the A. O. A. C. methods (13). Lignin, cellulose and other carbohydrates (by difference) were determined by the methods employed by Crampton and Maynard (11). Crude fiber (later referred to as "new crude fiber"), "new nitrogen-free extract" (by difference) and true fat were determined by the methods of Horwitt, Cowgill and Mendel (12). The cellulose determination was slightly modified by using a 50 ml. pyrex ignition tube for both digesting and centrifuging the sample, thereby eliminating the necessity of transferring the material from a 150 ml. round-bottom flask to a centrifuge tube. A temperature of 39° C. was used to incubate the lignin and new crude fiber samples. Linen (Butcher's) filtering cloth was used for filtering the new crude fiber sample because the solution passed through the linen much more rapidly than through various crucibles. For the final filtration, the linen and a beaker were weighed previously.

Rumen and total digestion coefficients were calculated by using (a) iron ratios and (b) lignin ratios. In addition, total digestion coefficients were calculated from the digestion trials. Bergeim (14) proposed a simplified method for the determination of food digestibility and utilization based on iron ratios. Iron oxide was added to the food and from the ratio of the amount of a given nutrient to the amount of iron in the food and feces, the percentage utilization was calculated. Dividing the fecal ratio by the food ratio and multiplying by 100 gave the percentage of the nutrient not digested. This percentage subtracted from 100 gave the percentage digested. Lignin was used in this investigation because of its highly inert character and the relatively large amount that is present in roughages. Knott and co-workers (15) illustrated the method of computation in the following formula:

$$100-\left(\frac{\text{per cent nutrient in feces}}{\text{per cent iron in feces}}\times\frac{\text{per cent iron in feed}}{\text{per cent nutrient in feed}}\right)100=\text{digestibility}$$

In this investigation *rumen* was substituted for *feces* to calculate rumen digestion coefficients and *lignin* was substituted for *iron* when the lignin ratio was being used.

A Beckman pH meter was used to obtain the pH values of the rumen contents at regular intervals during the day on the 10-, 20- and 30-pound levels. Values on a ration of hay, silage and concentrate were also recorded. Rumen and rectal temperature readings were taken with the pH readings.

Barrel circumferences and body weights were recorded for various animals in the experimental herd receiving different levels of alfalfa hay or green alfalfa. These data were usually taken every third day just before feeding in the afternoon.

TABLE 1

Composition of alfalfa hay, rumen contents and feces on the dry basis

Nutrient	Alfalfa hay			Rumen contents			Feces		
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Protein	16.2	18.1	16.3	13.5	14.8	11.1	11.7	12.5	10.3
Ash	6.8	7.4	7.0	9.5	9.9	9.4	10.9	9.7	9.3
Ether extract	2.6	2.2	1.7	2.9	2.5	2.3	4.4	4.1	2.2
True fat	0.51	0.69	0.67	1.09	0.83	0.80	0.47	0.34	0.53
Crude fiber	35.3	31.3	36.3	48.1	45.3	51.6	43.1	37.4	47.8
N-free extract	39.2	41.1	38.7	26.1	27.5	25.7	29.9	36.3	30.5
Lignin	15.7	15.8	17.7	29.9	30.8	33.2	31.1	29.4	32.3
Cellulose	36.3	29.2	34.2	36.2	34.6	39.5	33.3	28.9	36.4
Other carbohydrates	22.5	27.3	23.2	8.6	7.4	6.1	8.7	15.5	8.1
New crude fiber	53.2	48.1	53.9	73.6	71.8	81.9	73.6	67.9	73.9
New N-free extract	21.3	24.3	21.1	0.5	1.0	−3.2	−0.5	5.8	2.9
Iron	0.021	0.014	0.012	0.028	0.033	0.023	0.053	0.052	0.036

RESULTS

Analyses of the alfalfa hay, rumen contents and feces at the various levels of hay consumption are given in table 1. It will be observed that the combined values for lignin and cellulose approach the value for new crude fiber and that the values for true fat vary independently of variations in ether extract. The biological value of these determinations is readily observed by referring to table 3 and noting the greater digestibility of other carbohydrates and new nitrogen-free extract in comparison to the nitrogen-free extract. The lower digestibility of new crude fiber compared with crude fiber and the characteristic digestibility of the lignin and cellulose fractions may also be seen. True fat was much more highly digestible than ether extract.

TABLE 2

Rumen and total digestion coefficients on the basis of iron and lignin ratios

Nutrient	Coefficients of rumen digestion			Coefficients of total digestion		
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level
Iron ratios						
Dry matter	25.0	57.6	47.9	60.4	73.1	66.7
Protein	38.3	65.7	64.6	71.9	81.2	79.4
Ether extract	15.7	48.2	25.7	32.4	49.1	53.4
True fat	−58.9	46.3	37.3	64.0	89.7	74.9
Crude fiber	− 0.9	39.2	25.8	51.7	66.9	56.4
N-free extract	51.0	71.9	65.3	69.9	76.1	74.2
Lignin	−41.6	18.5	1.9	21.9	49.1	40.2
Cellulose	25.5	49.6	39.3	63.6	73.1	65.1
Other carbohydrates	70.9	88.3	85.7	80.6	84.7	88.7
New crude fiber	− 2.6	37.2	20.3	45.3	61.7	55.2
New N-free extract	97.6	94.2	100.0	99.1	92.5	91.2
Lignin ratios						
Dry matter	47.5	48.7	46.7	49.5	46.3	45.2
Protein	55.5	57.8	63.8	63.3	62.8	65.5
Ether extract	40.0	35.1	24.5	13.2	− 1.8	20.8
True fat	−13.8	33.2	34.8	53.4	71.2	57.9
Crude fiber	28.2	25.1	23.7	38.1	35.7	27.3
N-free extract	64.9	65.5	64.5	61.4	52.3	56.8
Cellulose	47.4	38.3	38.1	53.2	46.3	41.6
Other carbohydrates	78.0	85.4	84.9	80.3	69.4	81.1
New crude fiber	27.1	22.8	18.7	29.8	22.7	25.3
New N-free extract	98.0	93.3	100.0	98.9	86.3	84.9
Iron	27.5	−22.8	−2.6	−27.5	−100.0	−64.2

Table 2 presents the rumen and total digestion coefficients calculated on the basis of lignin and iron ratios. Most of the coefficients obtained for rumen digestion by means of lignin ratios closely approach the actual values for total digestion given in table 3. Although the calculated total digestion coefficients at the 10-pound level compare favorably with the actual coefficients for that level, the calculated values for the other two levels are con-

TABLE 3

The relation of rumen digestion to total digestion and the effect of the level of feeding

Nutrient	Rumen digestion, lignin ratio			Total apparent digestion*			Percentage of total occurring in rumen		
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level
Dry matter	45.7	48.7	46.7	56.3	55.8	46.9	84.4	87.3	99.5
Protein	55.5	57.8	63.8	68.9	68.4	64.7	80.6	84.5	98.6
Ether extract	40.0	35.1	24.5	17.2	13.4	−18.6	232.3	262.5	.....
True fat	−13.8	33.2	34.8	65.3	72.5	48.0	−17.5	45.8	72.5
Crude fiber	28.2	25.1	23.7	45.6	44.9	36.2	62.0	55.8	65.7
N-free extract	64.9	65.5	64.5	60.1	66.6	56.9	108.0	98.4	113.3
Lignin	0.0†	0.0†	0.0†	17.0	17.4	− 0.2	0.0	0.0	0.0
Cellulose	47.4	38.3	38.1	50.9	51.4	44.1	93.3	74.4	86.2
Other carbohydrates	78.0	85.4	84.9	85.0	84.7	80.2	91.8	100.9	105.8
New crude fiber	27.1	22.8	18.7	39.5	37.1	32.9	68.7	61.6	56.9
New N-free extract	98.0	93.3	100.0	86.6	90.0	72.8	113.2	103.7	137.4
Iron	27.5	−22.8	−2.6	−21.6	−68.6	−72.5	.....	.....	.....

* Determined by digestion trials.
† Lignin was assumed to be indigestible to facilitate calculations.

siderably lower than the actual coefficients. Calculations of rumen digestion with iron ratios gave highly variable results which were unusually low at the 30-pound level and somewhat high at the 20-pound level. The unusually high coefficients of total digestion obtained by using the iron index are explained by the negative iron balances in table 3.

The data presented in table 3 show the digestion coefficients of the various nutrients in the rumen on the basis of the lignin ratio, the total digestion coefficients obtained by digestion trials and the percentage of the total digestion that occurred in the rumen. The more simple carbohydrates represented by nitrogen-free extract, new nitrogen-free extract and other carbohydrates disappeared rapidly from the rumen. Digestible cellulose was largely removed while the feed was in the rumen while digestible crude fiber and new crude fiber were less than two-thirds removed before passage of the ingesta from the rumen. Of particular note among coefficients of rumen digestion is the negative coefficient of −13.8 per cent for true fat at the 30-pound level. This average value included one coefficient as low as −26.4 per cent.

The effect of the level of feeding on rumen and total digestion coefficients is shown in table 3. Increased hay consumption resulted in a decreased rumen digestion of protein and true fat and an increased digestion of ether extract, crude fiber and new crude fiber. Other nutrients did not vary directly with the level of feeding. Total digestion coefficients did not vary

TABLE 4

Effect of the level of feeding on rumen fill, live weight, and barrel circumference

Dry matter intake	Weight of rumen contents	Dry matter	Total dry matter	Weight of animal	Rumen fill as per cent of live weight	Barrel circumference
kg.	*kg.*	%	*kg.*	*kg.*	%	*cm.*
11.9	61.7	14.3	8.8	367	16.8	198
8.2	62.1	15.6	9.7	377	16.5	198
4.1	63.1	16.0	10.1	376	16.8	197
12.2	62.1	13.7	8.5	406	15.3	208
7.7	51.3	12.9	6.6	404	12.7	204
4.0	59.9	14.2	8.5	383	15.6	193
4.0	29.3*	14.5	4.2	368	8.0	191

* Rumen contents removed 24 hours after feeding.

appreciably when the level of feeding was increased from 20 to 30 pounds. The change resulted in a slightly lower digestibility of nitrogen-free extract, new nitrogen-free extract and true fat and an increased digestibility of ether extract and new crude fiber. All constituents were less digestible at the 10-pound level than at the higher levels.

The data presented in table 4 show that the amount and the character of rumen fill remained fairly constant regardless of the amount of hay consumed. The data in tables 4 and 5 illustrate that the body weight and

TABLE 5

Effect of the level of feeding alfalfa hay and green alfalfa on barrel circumference and live weight

Cow no.	D12				A10				D11		
Days fed	21	33	27	24	21	33	27	24	15	18	15
Lb. of hay	30	15	30	15	9	40	20	40	5	20	30
Barrel cir.*	248	239	243	234	222	227	225	233	206	214	225
Live weight†	.....	.....	.....	.....	.....	.....	.....	.....	978	1052	1139

Cow no.	A4			266			264	
Days fed	6	21	9	6	21	9	12	21
Lb. of hay	40	20	40	30	.....	30	30	15
Lb. of alfalfa‡	.....	100	.....	.....	100	.....	.....	.....
Barrel cir.*	240	231	240	218	212	219	241	232
Live weight†	1275	1222	1267	995	915	972	1153	1091

* Circumference in centimeters.
† Weight in pounds.
‡ Green alfalfa.

barrel circumference increase or decrease directly with variations in dry matter intake. The failure of these values to drop when the dry matter intake of the rumen fistula cow decreased from 11.9 to 4.1 kg. was the only exception. Feeding green alfalfa, however, resulted in distinctly lower values for both live weight and barrel size, although the dry matter intake remained the same.

In figure 1 the diurnal variations in rumen pH at the various levels of feeding are illustrated graphically. There was a steady decline in rumen pH during the first six hours after feeding. After that time, the pH rose

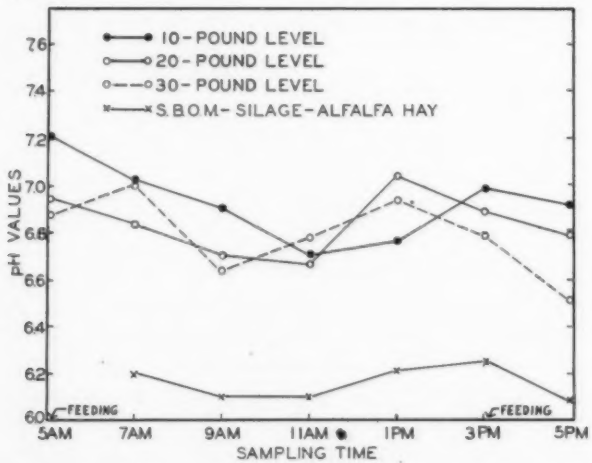

FIG. 1. Diurnal variations in the pH of rumen ingesta at various levels of alfalfa hay consumption.

until a maximum was reached just before feeding in the afternoon. This suggests a decrease in fermentation activities in the rumen about six hours following feeding. Rumen pH values on the alfalfa hay ration averaged 6.82 and were definitely higher than the pH values obtained on a ration of hay, silage and soybean oil meal. Variations of pH in different regions of the rumen at any one time never exceeded pH 0.3. Generally, the posterior region was more alkaline and the reticulum averaged 0.13 pH more alkaline than the rumen. These findings compare closely with the results reported by Monroe and Perkins (16). Rumen temperature was not affected by time after feeding or the level of hay consumption, although the highest values were obtained at the 30-pound level. The values ranged from 36.0 to 39.6° C. (mean 38.8° C.). If the few low values which resulted from water consumption are excluded, the mean rumen temperature was found to be 39° C. This value was 0.7° C. higher than the average rectal temperature. The difference was probably due to the more active fermentation in the rumen.

DISCUSSION

Value of iron and lignin ratios in determining digestion coefficients. Bergeim (14) reported that the iron ratio was a satisfactory method of determining food digestion and utilization in certain species but Knott and associates (15) reported that the iron ratio was not adaptable to digestion studies in the ruminant. Rathnow (23) used the iron index as a measure of digestion in the rumen and concluded that it served as a good indication of the rate of digestion. By this method he estimated that in three hours 32.1 per cent, in six hours 48.5 per cent and in nine hours 58.8 per cent of the dry matter in the rumen was digested. The data in tables 2 and 3 demonstrate the unreliability of the iron ratio as a measure of rumen digestion because the iron accumulated in the rumen in some instances and in others it passed from the rumen ahead of the ingesta.

The accuracy of the lignin index as a measure of rumen digestion is dependent upon the degree to which lignin is digested in the rumen. Any appreciable digestion of this substance in the rumen would result in low values for rumen digestion. The fact that the calculated coefficients for rumen digestion are high and in many instances approach or equal the total digestion coefficients indicate that lignin was not digested to any significant extent in the rumen and is, therefore, a suitable measure for rumen digestion. A comparison of the digestion coefficients for cellulose and new crude fiber further indicate that lignin is primarily digested after leaving the rumen. Digestible cellulose was largely removed while the ingesta was in the rumen but only about two-thirds of the digestible crude fiber was digested. The remaining one-third of the digestible crude fiber which was digested after leaving the rumen must have been lignin because most of the other major crude fiber fraction (cellulose) had been previously digested.

It is important to have some idea of the type and degree of error that would occur in event lignin were materially digested in the rumen. Calculations based on a comparison of cellulose and new crude fiber coefficients indicate that when the highest lignin digestibility coefficient was used (23.7 per cent) only 10 per cent of the lignin was digested in the rumen. By assuming a 10 per cent digestibility of lignin in the rumen, it was found that the percentage error in rumen digestion coefficients of highly digestible materials such as nitrogen-free extract, other carbohydrates and new nitrogen-free extract, would be nil and the coefficients for protein, ether extract and true fat would only be a few per cent. The percentage error for fibrous materials would be considerable with cellulose digestion coefficients being more nearly correct than those for the crude fiber fractions. These data indicate that the lignin ratio is a useful tool in ascertaining the digestibility of various nutrients in the rumen. Any errors that might occur would be negligible with the exception of coefficients for fibrous materials and in this instance the error would always be in the same direction, underestimation, and could be allowed for. This method of studying rumen activity should fill a need for some quantitative measure of rumen digestion and should be of considerable value in affording an understanding of the degree and significance of rumen digestion compared with the subsequent digestion that occurs during the passage of the ingesta through the remainder of the digestive tract.

Quantitative digestion in the rumen. It should be borne in mind that the digestion coefficients obtained by calculations with lignin ratios do not necessarily imply that the nutrients are in an absorbable state. Literally, they indicate the percentages of the various nutrients that pass from the rumen ahead of the lignin. In the case of cellulose and crude fiber the coefficients probably indicate completed digestion, whereas the more simple carbohydrates would probably be completely broken down and in solution. Other nutrients would seem to be sufficiently decomposed to allow digestion to be completed rapidly in the remaining portions of the digestive tract.

The carbohydrate materials included in the nitrogen-free extract, other carbohydrates and new nitrogen-free extract passed rapidly from the rumen and in most instances 100 per cent of the digestible portion of these materials had disappeared from the rumen by the time of sampling. It is probable that these more simple carbohydrates did not merely enter solution but that some were fermented by rumen bacteria before their passage to the other stomach compartments.

Digestible cellulose was largely removed by the fermentation processes of rumen micro-organisms with an average of 93.3 per cent being digested in the rumen at the 30-pound level of feeding and an average of 85.0 per cent being digested for all levels. Lignin was not digested to any material extent while in the rumen but was digested in variable amounts in the

remaining portions of the digestive tract. Csonka and co-workers (17) concluded from *in vitro* experiments that the degradation of lignin occurs in the stomach compartments of the cow and is not brought about by bacteria but possibly by some enzyme of the gastric juice. About two-thirds of the digestible crude fiber fractions and 90 per cent of the digestible dry matter were digested in the rumen.

Rumen digestion coefficients for ether extract were higher than total digestion coefficients indicating an accumulation of ether extractive material after passage of the ingesta from the rumen. That this material was non-fat in character is indicated by the fact that even though true fat was readily digested, a negative coefficient for ether extract might occur.

Rumen digestion coefficients for true fat indicate the probable products of the rumen digestion of cellulose and other carbohydrate materials. At the 30-pound level, at which bacterial activity would be greatest, there was an average increase of 13.8 per cent true fat in the rumen and one of the individual values included in this average represented a 26.4 per cent increase. Only two out of seven rumen digestion coefficients for true fat exceeded 20 per cent, while the total digestion of true fat averaged 66.1 per cent. Failure to find an increase in fat at the two lower levels may have been due to the continual passage of the products from the rumen and also to the fact that the samples were taken after the height of digestion had passed. These findings indicate the synthesis of fat by rumen microorganisms.

Supporting a synthesis of fat in the rumen are *in vitro* studies of cellulose fermentation by rumen bacteria by Woodman and Evans (5) and Pochon (6). Nicholson and Shearer (18) observed much volatile fatty acid in the rumen of animals studied at post mortem. Ritzman and Benedict (19) in discussing the metabolic stimulus of food stated, "in our judgment this average increase of 30 per cent noted with sheep confirms the striking increase noted with steers on a protein-poor, carbohydrate-rich maintenance ration, and suggests again the significance of the old view of Grouven that the path of absorption of carbohydrates in the ruminant is through the fermentative processes. These processes result in flooding the body with a large amount of the lower fatty acids which stimulate metabolism." Quittek (3) suggested the synthesis of fat in the rumen but his methods were not adequate enough to allow a definite conclusion and Krzywanek and Quittek (4) later concluded their results did not indicate a fat synthesis. The production of fatty acids from cellulose fermentation *in vitro* and the data supporting the formation of fat in the rumen suggest the possibility that the products of rumen digestion may play an important role in the physiology of ketosis in cattle. This subject has been discussed by Duncan and associates (20).

When the removal of the ruman contents was delayed until 24 hours after feeding, only a slight increase in rumen digestion coefficients was observed. This indicates that rumen digestion of the alfalfa hay was practically completed prior to 14 hours after feeding.

Effect of the level of feeding on rumen fill and digestion coefficients. The amount and character of the rumen fill remained fairly constant in spite of variations in hay consumption as great as 10 to 30 pounds. This finding is in agreement with the data of Ewing and Wright (21) who found that the weight and the dry matter content in the rumen of steers receiving from 1.66 to 3.84 kg. of dry matter daily did not differ significantly. These data indicate that the rumen fill was maintained at a constant level even though an increase in the level of feeding was always followed by an increase in barrel circumference and live weight. The change in barrel circumference was marked but was not perceptible to the eye. Feeding green alfalfa resulted in a decrease in body weight and barrel size even though the dry matter intake remained the same.

As the level of feeding increased there was a decrease in the digestion of protein and true fat in the rumen and an increase in the digestion of ether extract, crude fiber and new crude fiber. The rumen seems capable of handling highly variable quantities of roughage with equal efficiency. Total digestion coefficients for dry matter, protein, crude fiber, cellulose, lignin and other carbohydrates were not affected by increasing the level of feeding from 20 to 30 pounds of hay per day, while new crude fiber and ether extract were more digestible and only nitrogen-free extract, new nitrogen-free extract and true fat were less digestible. These results tend to support the conclusion of Watson and associates (10) that the plane of nutrition does not have any marked effect upon the digestibility of dried roughages. The marked decrease in digestibility which is often associated with a high plane of nutrition was not observed in this study. The low digestibility of all nutrients at the 10-pound level was attributed to the sub-maintenance level of nutrition. Watson and associates (10) and Forbes and co-workers (22) also observed a lowered digestibility on sub-maintenance rations.

Value of lignin and cellulose, new crude fiber and true fat in feed analysis. Partitioning the carbohydrate fraction of alfalfa hay into lignin, cellulose and other carbohydrates was found to be of greater value in estimating the biological value of hay than the usual division into crude fiber and nitrogen-free extract. Other carbohydrates averaged 21 per cent more digestible than the nitrogen-free extract, cellulose was 50.0 per cent digestible and lignin varied in digestibility from −5.1 to 23.7 per cent.

The values obtained by the enzymatic method for crude fiber gave a much sharper distinction between the highly digestible and poorly digestible fractions of carbohydrate than the values obtained by the common crude

fiber method. By the common method the difference in the digestibility of crude fiber and nitrogen-free extract was only 19.5 per cent compared with 49 per cent by the enzymatic method. Practically 100 per cent of the new nitrogen-free extract disappeared in the rumen compared with 65 per cent of the nitrogen-free extract and 83 per cent of other carbohydates. The new nitrogen-free extract was slightly more digestible than other carbohydrates indicating that the material—other than cellulose or lignin—present in the new crude fiber fraction was poorly digestible in character. The enzymatic method for crude fiber requires somewhat less labor than the lignin and cellulose determinations but more time is required to obtain the results because an 8-day incubation period is necessary in comparison to a 12-hour incubation period for lignin.

The true fat values indicate greater significance than the ether extract values. True fats were found to be 66.1 per cent digestible, while only 8.6 per cent of the ether extract was digestible. Attention should be directed to the fact that the methods generally employed for the determination of fat in feed materials do not completely remove the fat and allied substances. In view of the low values obtained in this study for true fat, it is suggested that additional methods should be investigated for the complete extraction and determination of fat in feeding stuffs.

SUMMARY AND CONCLUSIONS

One rumen fistula and five normal Holstein cows received alfalfa hay in amounts of 10, 20, or 30 pounds per day. Rumen contents were removed 14 hours after feeding on six occasions and 24 hours after feeding on one occasion. A total of 11 digestion trials were conducted. The chemical analyses which were made on the hay, rumen contents and feces were moisture, protein, ash, ether extract, true fat, crude fiber, nitrogen-free extract, lignin, cellulose, other carbohydrates (by difference), crude fiber by an enzymatic method, new nitrogen-free extract (by difference), and iron.

The use of lignin ratios is proposed as a method of studying quantitative digestion in the rumen. This method is useful in studying the significance of rumen digestion as related to subsequent digestion in the remainder of the digestive tract and is relatively accurate. Iron ratios are not reliable for determining rumen or total digestion coefficients.

An average of 90 per cent of the digestible dry matter passed from the rumen before 14 hours after feeding. Digestible nitrogen-free extract, other carbohydrates and new nitrogen-free extract were completely removed from the ingesta during rumen digestion. Eighty-five per cent of the digestible cellulose had been digested at the time of sampling. Lignin did not appear to be appreciably digested in the rumen even though total digestion ranged up to 23.7 per cent but digestible crude fiber was two-thirds digested

in the rumen. There was an accumulation of ether extractive substances after the ingesta passed from the rumen.

True fat was not digested to any extent in the rumen and at the 30-pound level of feeding there was an actual increase in true fat. These findings suggest the synthesis of fat in the rumen by micro-organisms from such materials as cellulose and other carbohydrates.

Rumen pH on a ration of alfalfa hay was neutral or slightly acid (average pH 6.82) and the maximum acidity was reached about six hours after feeding. A mixed ration gave lower rumen pH values. Rumen temperature approximated 39° C.

The character and amount of rumen fill were not affected by the amount of feed consumed, although the body weight and barrel circumference varied directly with hay consumption.

As the plane of nutrition increased the rumen digestion of protein and true fat decreased while that of ether extract, crude fiber and new crude fiber increased. Total digestion coefficients for nitrogen-free extract, new nitrogen-free extract and true fat decreased as the level of feeding increased from 20 to 30 pounds. The other nutrients were either not affected or increased slightly in digestibility. The low digestibility of all nutrients at the 10-pound level was attributed to the sub-maintenance level of nutrition.

The separation of the carbohydrate fraction into lignin, cellulose and other carbohydrates is a better index to the biological value of alfalfa hay than the present division into crude fiber and nitrogen-free extract. The values obtained by the enzymatic determination of crude fiber gave a much sharper biological distinction between the two carbohydrate fractions than the standard method. Inasmuch as true fat averaged 66.1 per cent digestible and ether extract only 8.6 per cent, it is evident that the true fat determination is of greater biological significance than the ether extract determination.

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A RAPID COLORIMETRIC METHOD FOR THE DETERMINATION OF LACTIC ACID IN MILK

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Many methods for determining lactic acid in biological fluids have been reported. Several have been applied successfully to dairy products. Most of these methods, however, are too time consuming to be used regularly in the control laboratory. A search was made, therefore, for a method which was both rapid and reliable: A method using a simple procedure for the removal of interfering substances; a simple oxidation procedure; and a simple means of estimating the quantity of lactic acid present.

The method Mendel and Goldscheider (5) used for determining the lactic acid in blood seemed to be the most promising, but when applied to milk gave unreliable results. Many variations were tried, one of which is reported below. The method for removing interfering substances is almost identical with that described by Troy and Sharp (7). Their work on precipitation procedures was repeated to determine the most satisfactory technique to be used in conjunction with the colorimetric method, but no change was found necesssary.

PROCEDURE

Weigh 5 grams of milk in a 50 ml. volumetric flask. Add 30 ml. of cold water and mix. Dilute a portion of a 25 per cent solution of copper sulphate 1:3 and add dropwise with agitation 2–8 drops of the dilute solution to the water and milk mixture until precipitation occurs. Warm the flask in hot water until the contents reach 45° C. Add 6 ml. of 25 per cent copper sulphate solution and mix thoroughly by rotation. Hold the contents of the flask at 45° to 47° C. for 8 to 10 minutes. Next loosen any curd particles which may adhere to the flask with a bent wire. Add 6 ml. of calcium hydroxide suspension. This suspension is prepared by slaking 300 grams of the best quality calcium oxide using 1400 ml. water and working through a 24 mesh screen after 30 minutes standing. Before mixing the lime with the other constituents in the flask, water should be added to bring the contents to exactly 50 ml. Then mix until the contents are homogeneous and allow to stand at 45° to 47° C. for 10 minutes. Cool to 25° C. and filter, discarding the first few ml. of filtrate.

Place 1.0 ml. of the filtrate in a thoroughly clean and dry test tube. Hold the test tube in ice water and allow 5.0 ml. of cold concentrated sulphuric acid (arsenic free) to run down the side wall of the test tube. Mix gently while cooling. Place mixture in boiling water for exactly 5 minutes.

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Cool in ice water for three. Add 4 drops of a 0.1 per cent solution of veratrole in water and mix. Hold in ice water for 60 minutes and compare colors with artificial or natural standards.

Natural standards may be prepared according to Hillig (3). Dissolve 106.6 mg. of purified lithium lactate in 100 ml. of distilled water. 1.0 ml. of this solution contains the equivalent of 0.1 per cent lactic acid. Consequently, to prepare a 0.01 lactic acid standard, 5.00 ml. of this solution is diluted to exactly 50.0 ml. and mixed. One ml. of this dilution is then placed in a test tube and 5 ml. of concentrated sulphuric acid added. The mixture is held in boiling water 5 minutes and cooled in ice water 3 minutes. Four drops of a 0.1 per cent solution of veratrole is then added and the color observed after 60 minutes. Additional standards may be made by diluting the original lithium lactate solution as required.

Artificial standards which are fairly satisfactory may be prepared according to the method of Nordbö (6). Mix 25.0 ml. of a 0.01 per cent solution of fuchsin in water and 4.75 ml. of a 0.01 per cent solution of Tropaolin 000. (The mixture must be diluted immediately for a precipitate forms on standing.) One tenth ml. of this mixture diluted to 50.0 ml. of solution is equivalent to approximately 0.005 per cent lactic acid; 0.25 ml. diluted to 50 ml. is equivalent to approximately 0.01 per cent and 0.9 ml. diluted to 50 ml. is equivalent to 0.02 per cent. Nordbö's standards are only suitable for concentrations below 0.03 per cent. Above this percentage, the yellow color increases in intensity. Consequently, for a 0.05 per cent lactic acid standard, it is necessary to add 0.4 ml. of .01 per cent Tropaolin and 1.8 ml. of .01 per cent fuchsin to 47.8 ml. of water; for a 0.07 per cent standard, 1.0 ml. of Tropaolin and 2.0 ml. of fuchsin to 47.0 ml. of water; and for a 0.10 per cent standard, 2.0 ml. of Tropaolin and 2.0 ml. of fuchsin to 46.0 ml. of water. These artificial standards should be checked against natural standards before using.

DISCUSSION

According to the authors, the original test which was applied to blood is accurate to $\pm$ 0.004 per cent between 0.001 and 0.05 per cent (5) and 1 part in 400,000 lactic acid could be detected. Mendel (4) later made two improvements which he stated made possible the detection of one part in 1,000,000. However, when applied to milk, the original test was sensitive to 0.01 per cent and had a limited degree of accuracy ($\pm$ 0.04 per cent). With the modification outlined above, the test is sensitive to 0.005 per cent and accurate to $\pm$ 0.004 per cent between the ranges of 0.005 and 0.12 per cent lactic acid providing the procedure is closely followed.

Mendel (4) has pointed out that small changes in the concentration of the sulphuric acid may lead to inaccurate results. A difference of 2 to 3 per cent in the concentration may result in as much as a 15 to 20 per cent

error. It is wise, therefore, to check frequently the color of the artificial standards. In this respect, it should be noted that the individual analyst should try several different quantities of sulphuric acid (*i.e.*, 4.5, 4.75, 5.0 ml. etc.,) in the preparation of natural standards in order to ascertain the exact amount to add to the 1.0 ml. filtrate to obtain the greatest degree of color development for each batch of sulphuric acid. Derviz (1) describes a method for purifying and storing sulphuric acid so as to maintain a constant concentration.

Mendel (4) makes the recommendation that after the mixture of acid and filtrate has been held in boiling water five minutes, it be cooled 2 minutes in ice water, veratrole added, and set at 25° C. After 20 minutes, the color may be compared with standards similarly treated. This method may be employed whenever results of great accuracy are not desired.

Mendel and Goldscheider (5) used 0.1 ml. of a 0.125 per cent veratrole in 99.8 per cent alcohol. Other investigators suggested 0.05 ml. of a 1:800 solution in 96 per cent alcohol (6), 0.1 ml. of 20 per cent veratrole solution in glacial acetic acid (1) and 0.1 ml. of 20 per cent in absolute alcohol (2). These were all tried, but none gave as satisfactory results as 4 drops of a 0.1 per cent solution in water. Various solutions of guaicol, hydroquinone, and Schiffs reagent were also tried and considered unsatisfactory.

Although the original method called for a 4 minute heating period, 0.5 ml. filtrate and 3.0 ml. of conc. H_2SO_4, it was found that 5 minute heating period, 1.0 ml. filtrate and 5.0 ml. of concentrated sulphuric acid gave more uniform results.

The same substances interfere with this method as those Troy and Sharp (7) found to interfere with theirs. Formaldehyde, overneutralization, rancidity, sucrose, and products resulting from the heating of milk at or near the boiling point for one half hour or longer resulted in high values. Mendel and Goldscheider state, however, that acetone, β-oxybutyric acid, acetic acid, urea, uric acid, creatin, creatinin, glycocol, alanine, and propionic acid, do not interfere with the color development.

Traces of organic matter interfere with proper color development and consequently the test tubes must be thoroughly clean and kept stoppered as much as possible during the determination.

When greater rapidity of testing is required, the precipitate of proteins, lactose and fat, may be centrifuged. In this case, cream test bottles (18 gram body, 9 gram neck, graduated in 1 per cent between 0 and 55 per cent) were selected from stock and graduated to contain 50.0 ml. at 20° C. The milk was weighed in these bottles which, after precipitation and cooling, were placed in a Babcock tester and centrifuged 4 minutes. The centrifugate was then decanted through a fluted filter, the first few ml. of filtrate being discarded.

SUMMARY

A rapid colorimetric method for the quantitative estimation of lactic acid in milk is described. The steps involved are precipitation of fat, lactose and protein with copper sulphate and calcium hydroxide, filtering, adding concentrated sulphuric acid (arsenic free) to the filtrate, heating, cooling, and adding veratrole. A red color develops on standing which is in proportion to the amount of lactic acid present.

The test is sensitive to about 0.005 per cent and is accurate to $\pm$ 0.004 per cent between the ranges of 0.005 and 0.12 per cent lactic acid.

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GROWTH OF CERTAIN ORGANISMS OF IMPORTANCE TO THE DAIRY INDUSTRY ON NEW STANDARD MILK AGAR*

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The medium used for making counts on milk and other dairy products, which became official July 1, 1939, has created considerable interest as to the effect it will have on dairy control practices. This paper deals with the relative amount of growth of certain pure cultures of bacteria on the old and new standard agars together with the total number of colonies as determined with these two agars on milk samples collected aseptically from an experimental dairy herd. The animals in the herd were divided into three groups: Those known to be free of streptococcic mastitis; those suspected of the disease because of high leucocyte counts; and those with clinical evidence of the disease and with long chained streptococci in the incubated milk.

RESULTS WITH PURE CULTURE STUDY

The pure cultures used were a series of pathogenic and non-pathogenic Gram-positive cocci and bacilli of importance to the dairy industry. These organisms were examined carefully for purity and identified. The "pyogenic" streptococci were all typical of the group as outlined by Sherman (1). The two cultures of *S. scarlatinae* (? name) were from clinically typical severe cases of scarlet fever and were of proved toxicity. The 53 cultures of *S. agalactiae* were isolated from chronic cases of bovine mastitis from twenty cows in one herd. These cultures had been isolated from one to nine months before being used. The seven, "Animal Pyogenes," streptococci were obtained from equine infectious processes. The pure cultures of non-pathogenic organisms were obtained from various workers in the field of dairy bacteriology. These cultures had been identified by others and were re-examined only to determine purity before being used in this study.

Sugar-free meat infusion agar slants were used as a medium for the cultivation of pure cultures used in this experiment. Within 24 hours after inoculation all cultures had grown enough to permit the preparation of saline suspensions which could be standardized as to turbidity. From these suspensions, serial dilutions were made and plated in duplicate. One set was poured with old standard agar (2) and the other with new standard agar (3). The plates were then incubated at 37° C. for 48 hours. Counts were made with: (a) the unaided eye; (b) Quebec colony counter, and (c) the 10× and 30× wide field binoculars.

The ability of the organisms to develop observable colonies on the two agars is clearly shown in table 1. In case of certain of the organisms, com-

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TABLE 1

Result of pure culture studies

Organism	Strains	Colony detection on	
		New medium	Old medium
S. scarlatinae	2	Easily countable Quebec counter	None countable, 30× magnification
S. agalactiae	53	All easily countable Quebec counter	5—countable Quebec counter 20—10× magnification 10—30× magnification 18—none countable
"Animal Pyogenes" Streptococci	7	All easily countable Quebec counter	None countable Quebec counter
S. durans	1	Easily countable	Not countable Quebec counter
S. lactis	1	Easily countable Quebec counter	Not countable Quebec counter
S. fecalis	1	Easily countable Quebec counter	Not countable Quebec counter
S. zymogenes	1	Easily countable Quebec counter	Not countable Quebec counter
L. bulgaricus	4	1 countable 3 non-countable	None countable
L. acidophilus	2	Easily countable	1—not countable 1—difficultly countable
S. thermophilus	1	None countable	Not countable
S. liquefaciens	5	4 countable 1 not countable	4—pin-point not ordinarily countable 1—not countable
L. casei	3	1 countable 2 countable with difficulty	Not countable

plete inability to develop in the new medium is unquestionably due to lack of necessary nutrients. With other organisms, *S. agalactiae* for instance, ability to develop on the old medium apparently depends on strain variation. In all instances, with the exception of the culture of *S. thermophilus*, one or more strains of each grew well on the new medium. The addition of such nutrients as glucose, skim-milk and possibly tryptone makes a more suitable medium for the growth of these organisms.

RESULTS OF PLATE COUNTS ON MILK

The milk samples were obtained from the following groups of experimental cows: (a) 26 cows known to be free of mastitis; (b) 16 cows suspected of mastitis; (c) 26 cows known to have mastitis. All cows included in each group had been so classified for a period of at least three months and some had been grouped, as above described, for a period of 24 months. Equal quantities of milk, as far as practicable, were collected aseptically from each quarter and taken immediately to the laboratory for analysis.

A summary of the results on milk samples is given in table 2. From an examination of these data it is evident that the quality of the milk from the

TABLE 2

Milk sample studies

Group of cows	Mastitis free		Mastitis suspects		Mastitis positive	
Medium	New	Old	New	Old	New	Old
Number of animals in group	26	26	16	16	26	26
Log. Ave. count	126	47	912	389	4,470	2,690
Minimum count	3	1	50	30	300	10
Maximum count	17,500	12,000	4,800	4,700	896,000	634,000
Median count	77	35	1,145	665	4,350	2,200

three groups is quite different. The minimum, median and maximum counts on the three groups of cows has been determined. In all instances the minimum and maximum counts from the same animal were consistently higher in the new medium. The median count for each group of samples indicates that the new medium gives counts about double those obtained on the old medium.

In addition to the above described examples of the superiority of the new agar, it is of interest to compare the results obtained by using "new," "old" and meat infusion blood agars in a study of an epidemic of septic sore throat involving approximately forty cases. The total count per ml. milk from the involved quarter of the animal responsible for the epidemic as plated on the "old," "new" and blood agars was 5,800, 149,000, and 115,000, respectively. On blood agar 105,000 or 91 per cent of the total number of colonies counted were beta-hemolytic streptococci which were later found to belong to Lancefield group A.

CONCLUSIONS

It is clearly shown by this investigation that the pyogenic streptococci which were studied responded well to the new medium. The other streptococci and lactobacilli did not show the same degree of response; but growth was definitely improved in the case of most, but not all, of the organisms.

Milk from three groups of cows, in a mastitis control project, individually and collectively, showed definitely, higher plate counts when the new medium was used.

When the milk from an animal responsible for an outbreak of septic sore throat was plated on the new medium, there was a higher count than when blood agar was used. However, the use of blood agar is essential in the detection of such organisms and should be used in all such investigations.

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A STUDY OF THE REDUCING SYSTEM OF MILK*

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Since the introduction of the chemical method for the determination of ascorbic acid in biological materials, numerous studies have been conducted dealing with the ascorbic acid content of milk. However, to date, little attention has been given to the possibility that milk may contain other reducing substances, which, under certain conditions, may exert an influence similar to that exhibited by ascorbic acid. This study was conducted, therefore, with the view of determining the total reducing power of milk as measured by potassium iodate titration procedure, and then ascertaining the proportion of this power which is contributed by ascorbic acid.

Two reducing substances which are present in many biological systems and which have attracted considerable attention of research workers are ascorbic acid and glutathione. Fresh raw milk contains usually from 15 to 30 mg. of ascorbic acid per liter (5, 16, 11, 2), which exists in its entirety in the reduced form (9). Age, aeration, sunlight, and contamination with copper cause a destruction of the ascorbic acid in milk (16, 15, 14, 4), whereas heat, per se, exerts only a negligible effect upon the ascorbic acid content (2, 4).

The presence of glutathione in milk is disputed. Martini (10) reports that milk contains about 8 mg. per cent, but Jackson (6) Gould and Sommer (3) and Josephson and Doan (8) were unable to secure positive nitroprusside tests on normal raw milk. However, treatment of raw milk with sodium cyanide results in positive nitroprusside reactions (3, 6).

Jackson (7) observed that fresh, anaerobically drawn milk exhibited much greater reducing power than did the same milk if exposed for a few minutes to the atmosphere. Whether this indicates the presence of a strong reducing substance in milk which is quickly oxidized by atmospheric oxygen, or whether the change is merely due to the diffusion of oxygen into the milk has not been shown.

In addition to ascorbic acid, and probably glutathione or cysteine, the flavin system of milk is also of importance in connection with oxidation-reduction phenomena (4, 7). Jackson found that when whey concentrates were added to milk, the decoloration of methylene blue was greatly hastened.

EXPERIMENTAL METHOD

The methods used were those suggested by the work of Woodward (17) and DeWitt and Sure (1). The KIO_3 titration procedure of Woodward

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and Fry (18) was utilized to measure that power which may for convenience be called the "total reducing power" of the milk. The method is known to measure both the glutathione and the ascorbic acid content and has been used for that purpose (1, 17). The ascorbic acid was determined by the 2, 6-dichlorophenolindophenol titration procedure adapted to milk by Sharp (11).

The KIO_3 method of Woodward and Fry (18) for glutathione in blood was modified only insofar as to permit the titration of a more concentrated filtrate. Forty milliliters of milk were diluted with 50 ml. of water, and the proteins precipitated with 10 ml. of molar sulfosalicylic acid solution. Ten milliliters of the filtrate were titrated with 0.001N KIO_3 solution. The filtrate possessed a pH well under 2. Comparisons of this slightly modified procedure with the original micro procedure were made on fresh raw milk, and close agreement between the methods was secured.

The use of iodoacetate to block out the -SH groups was made, in general, on the basis of the work of Schultze, Stotz, and King (12).

Milk used for the study was in most instances obtained directly from the College herd. It was collected in such a manner as to eliminate contamination with copper. The heating of the milk was conducted in a glass flask suspended in a boiling water bath. Temperatures were controlled well within the limits of 0.5° C.

RESULTS

Comparison of total reducing capacity with that contributed by ascorbic acid: The titration of milk with the 0.001N solution of KIO_3 gave values which were surprisingly high when calculated as glutathione or ascorbic acid. These results are presented in table 1, columns (1) and (2). These values range approximately from 119 to 192 when calculated as mg. of glutathione per liter, or from 34 to 55 when expressed as mg. of ascorbic acid per liter.

That the KIO_3 values are decidedly higher than would be the case if the ascorbic acid alone were responsible may be seen by comparing columns numbered two and three. The actual ascorbic acid values, as determined by titration with 2, 6-dichlorophenolindophenol, range only from 11.8 to 21.9 mg. per liter, as contrasted to the approximate range of 34–55 mg. per liter obtained from the KIO_3 titration. As shown in the final column, the ascorbic acid usually accounted for only 30–40 per cent of the total reducing action of the milk as determined with KIO_3, although in certain trials the ascorbic acid accounted for from 40 to 60 per cent of the total reducing power.

Influence of sunlight and copper on the total reducing capacity: Further evidence to show that the KIO_3 titration values are due only partially to the ascorbic acid was obtained when fresh raw milk was exposed either to

TABLE 1

Comparison of 2, 6-dichlorophenolindophenol and KIO_3 values when calculated as ascorbic acid and glutathione

Sample	KIO_3 values		Actual ascorbic acid value*	Per cent actual ascorbic acid value of total KIO_3 value
	As GSH (1)	As ascorbic acid (2)	(3)	(4)
	mg./liter	*mg./liter*	*mg./liter*	
1	134.0	38.29	12.07	31.52
2	118.9	33.97	11.83	34.82
3	144.2	41.20	12.51	30.36
4	128.8	36.80	13.04	35.43
5	174.8	49.94	18.56	37.16
6	191.7	54.77	21.93	40.04
7	174.9	49.97	15.89	31.80
8	165.7	47.34	17.94	37.90
9	158.0	45.14	17.48	38.72
10	145.7	41.63	15.75	37.83
11	164.9	47.11	18.90	40.12
12	121.0	34.63	15.79	45.60
13	145.7	41.63	21.15	50.80
14	126.3	36.08	21.09	58.45

* Determined with 2, 6-dichlorophenolindophenol.

sunlight for one hour or treated with 2 p.p.m. of copper for three hours. The KIO_3 values secured before and after such treatment and their comparison with the correct indophenol values are shown in table 2.

The KIO_3 results, when expressed either as glutathione or ascorbic acid show rather high values following the treatment of the milk with the sunlight or with the copper. Contrasted with this is the fact that these two agents, *i.e.*, sunlight or copper, destroy the ascorbic acid. In fact, they are utilized for this purpose in order to determine the so-called residual titer, or blank, in the indophenol titration. The ascorbic acid values shown in the table have been corrected for this residual titer.

The data in table 2 show further that the decrease in KIO_3 values caused by sunlight or copper is doubtless due entirely to the ascorbic acid itself, and not to any appreciable extent to the other reducing substances which are responsible for the remainder of the KIO_3 titer. This may be observed by comparing the columns numbered six and seven. The values in these columns show close agreement as indicated by the differences in column seven.

Use of iodoacetate: If the KIO_3 titer in milk is partly due to –SH linkages, iodoacetate should block out such groups and thus decrease the titer accordingly. Consequently, trials were conducted in which the milk was treated with sufficient iodoacetate to make a 0.1 molar concentration in the milk. Following the addition of the iodoacetate, the milk was stored at 4° C. for one hour. KIO_3 and indophenol titrations were made on the milk before and after treatment. The results of four trials are presented in table 3.

TABLE 2

*The influence of sunlight and copper on the KIO_3 and 2, 6-dichlorophenolindophenol values of milk**

Treatment of sample	KIO_3 values						Actual ascorbic acid values	Difference (7) from (6)
	As GSH			As ascorbic acid				
	Original (1)	After (2)	Difference (3)	Original (4)	After (5)	Difference (6)	(7)	
	mg./liter	*mg./liter*	*mg./liter*	*mg./liter*	*mg./liter*	*mg./liter*	*mg./liter*	*mg./liter*
Sunlight	134.0	92.0	42.0	38.29	26.29	12.00	12.07	+0.07
"	118.9	69.0	49.9	33.97	19.71	14.26	11.83	−2.43
"	128.8	69.0	59.8	36.80	19.71	17.09	13.04	−4.05
"	121.2	69.0	52.2	34.63	19.71	14.91	15.79	+0.88
Copper	174.9	105.8	69.1	49.97	30.23	19.74	15.89	−3.85
"	165.7	109.7	56.0	47.34	31.34	16.00	17.94	+1.94
"	158.0	92.8	65.2	45.14	26.51	18.63	17.48	−1.15
"	145.7	79.0	66.7	41.63	22.57	19.06	21.15	+2.09
"	145.7	92.0	53.7	41.63	26.29	15.34	15.75	+0.41
"	164.9	92.0	72.9	47.11	26.29	20.83	18.90	−1.93

* Milk treated with bright sunlight for one hour or with 2 p.p.m. of copper for three hours.

TABLE 3

The influence of iodoacetate on the KIO_3 and 2, 6-dichlorophenolindophenol titration values of milk

Trials	KIO_3 values as glutathione		2, 6-dichlorophenolindophenol values as ascorbic acid	
	Before treatment	After treatment	Before treatment	After treatment
	mg./100 ml.	*mg./100 ml.*	*mg./liter*	*mg./liter*
1	13.43	10.20	11.38	12.74
2	15.43	10.58	17.35	17.51
3	14.95	11.50	15.52	15.52
4	12.63	11.88	21.09	19.50

These data show that the iodoacetate treatment slightly lowered the KIO_3 values, whereas it had no definite effect upon the ascorbic acid content. The KIO_3 values decreased on the average, to an extent equivalent to approximately 3 mg. of glutathione per 100 ml. milk. Numerous other trials gave similar results.

Use of zinc dust: To determine the total glutathione content of blood, Woodward and Fry (17) added zinc dust to the sulfosalicylic acid filtrate. This procedure reduced the oxidized form of glutathione so that it could be measured by the KIO_3 titration. On this basis, the same procedure was utilized in this study to ascertain if the zinc dust treatment would increase the KIO_3 values of milk. Results of four trials are presented in table 4.

TABLE 4

The influence of zinc dust treatment on the KIO_3 values

Sample	KIO_3 values as glutathione	
	Before reduction	After reduction
	mg./100 ml.	*mg./100 ml.*
1	14.18	14.58
2	15.33	14.95
3	12.65	13.43
4	16.33	16.10

These results show the zinc dust to have no definite effect on the KIO_3 values. The values were substantially unaltered by the reduction, indicating that milk contains no substance in the oxidized form which may be reduced by the method and exposure time used in this experiment.

Influence of heat on the total reducing capacity: When milk has been heated sufficiently high the KIO_3 values are lowered, whereas the ascorbic acid content remains practically unchanged. This is illustrated by figure 1.

This graph shows that the KIO_3 values, (expressed as mg. glutathione per 100 ml.) undergo marked decreases in milk which has been heated to 80° C. or above. The value was practically unchanged, however, when the

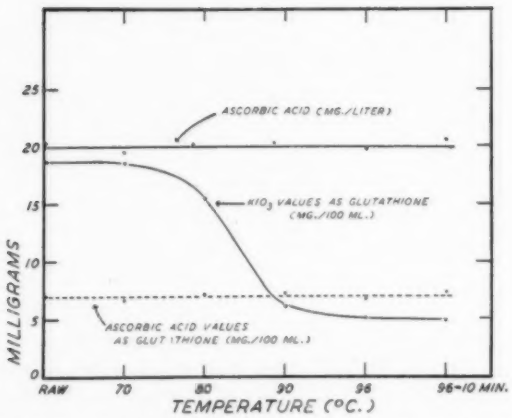

FIG. 1. The influence of heat on the KIO_3 values and ascorbic acid of milk.

milk was heated to 70° C. The largest drop in the KIO_3 values occurred between 80° C. and 90° C., and the higher temperature of 96° C., or 96° C. for ten minutes, caused only a relatively small further decrease. The graph shows also, that the ascorbic acid content as determined by 2,6-dichlorophenolindophenol remains substantially unaltered by the heat treatment.

The fact that the heat treatment had no definite effect upon the ascorbic acid indicates that the changes which occur in the KIO_3 values are due to decomposition or rearrangement of the other reducing substances involved. Further, since temperatures above 90° C. brought only slight further decreases in the KIO_3 values, the indications are that the bulk of the reducing material which is being acted upon has undergone change, and that the titer exhibited by this heated milk is due practically entirely to ascorbic acid alone. That this is true is illustrated in figure 1 by the line showing the ascorbic acid content expressed in its equivalent as mg. of glutathione per 100 ml. of milk. It may be observed that the ascorbic acid somewhat more than accounts for the KIO_3 values of the milk heated to 90° C. or above.

DISCUSSION

The data assembled in this experiment show conclusively that milk contains reducing substances, other than ascorbic acid, which are measurable by potassium iodate. These reducing substances contribute varying proportions of the total reducing capacity of milk, ranging from 40 to 70 per cent of the total. In all cases encountered in this and subsequent studies, the total of the KIO_3 values have always exceeded that which is contributed by the ascorbic acid alone. Conclusions should not be drawn at the present time as to what substance or substances are responsible for this reducing action of milk, but the proteins of the milk serum may be involved.

Sulfhydryl linkages are not found in milk, consequently it may be logically assumed that neither cysteine, glutathione, nor thioneine contribute to the KIO_3 values. If glutathione were involved, the treatment of the milk with copper would have affected the titration values of the non-ascorbic acid portion of the reducing materials. Although lactose is a reducing sugar, it is unlikely that it contributes to the reducing action, especially at the low pH at which the KIO_3 determination is conducted.

It is interesting to note that the reducing power of the substance responsible for the KIO_3 reducing ability of milk in excess to that contributed by the ascorbic acid is destroyed by heat at temperatures which cause the liberation of volatile sulfides from milk (3). This may be a coincidence. However, if proteins are involved, then consideration should be given the possibility that the substance contributing to the heat labile sulfides is also responsible for the bulk of the KIO_3 titer.

CONCLUSION

Potassium iodate titration values of milk are greater than they should be if ascorbic acid alone were responsible.

The material in milk responsible for the iodate reducing power of milk in excess of that caused by the ascorbic acid is unaffected by sunlight or copper under the conditions of this experiment, but is slightly affected by treatment of the milk with iodoacetate.

KIO_3 values of milk are not changed by zinc dust reduction of a sulfosalicylic acid filtrate.

The potassium iodate values of milk are lowered when milk is heated to 80° C. or above. This lowering is likely due to destruction or rearrangement of the non-ascorbic acid portion of the reducing materials.

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THE DISAPPEARANCE OF ADDED GLUTATHIONE IN MILK*

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Previously (1), the author observed that when 5 mg. of glutathione were added to milk, it was not detected by the nitroprusside test after a 15-minute incubation period. However, the same amount of glutathione added to milk which had been heated to 76° C. or above produced a positive nitroprusside reaction. This indicated the possibility that raw milk destroys glutathione, whereas milk which has been heated sufficiently high fails to exhibit this destructive property. Consequently, this study was conducted to determine quantitatively the disappearance of glutathione when added to raw and heated milk.

Oberst (2) found reduced glutathione to disappear when added to solutions containing egg albumin, casein and blood serum. He found both reduced and total glutathione content to decrease rapidly during the first 2–3 hours of incubation when added to whole blood. When glutathione was added to blood serum, the reduced glutathione concentration changed from 50.6 mg. per cent to 18.4 mg. per cent in 15 minutes, whereas the total glutathione content was reduced from 55.2 mg. per cent to 36.2 mg. per cent during the same period. Oberst suggests that the reduction of glutathione in protein solutions is due to chemical reaction, the glutathione combining with the protein so as to inactivate the sulfhydryl group.

EXPERIMENTAL PROCEDURE

Milk used for this experiment was secured directly from disease-free cows at the college farm. Porcelain pails were used to collect the milk in order to eliminate metal contamination. The milk was held at 4° C. until used.

Glutathione (Pfanstiehls) was added to the milk at the rate of 50 mg. per cent and was thoroughly incorporated by stirring the milk frequently during the first 15 minutes of the incubation period. The glutathione determination, conducted essentially by the method of Woodward and Fry (3) as outlined in an earlier paper (4), was made on the milk after 15 minutes, 1 hour, 2 hours and 4 hours of incubation at 20° C.

Heating of the milk was conducted in a round bottom flask suspended in a boiling water bath. Samples of milk were withdrawn by means of a glass-tube siphon. Temperatures were accurate within $\pm$ 0.5° C.

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RESULTS

Addition of glutathione to raw milk: Three samples of milk were secured from individual cows. Glutathione was added at the rate of 50 mg. per cent. The rate of disappearance of the reduced glutathione is illustrated by figure 1. The results are expressed as the actual glutathione present;

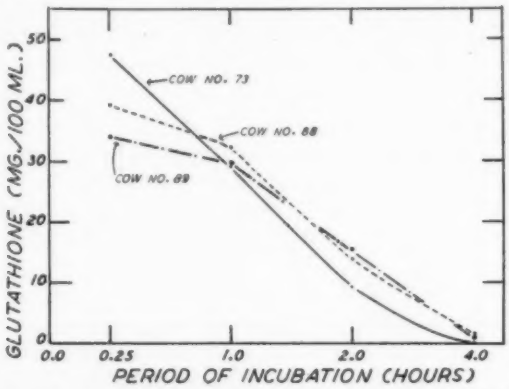

FIG. 1. The disappearance of reduced glutathione in raw milk (GSH added at rate of 50 mg. per cent).

consequently corrections are made in every case for the original KIO_3 values of the milk.

Figure 1 shows the loss of glutathione in milk to proceed at a rapid rate. Following the 15-minute interval the glutathione values are considerably below the 50 mg. level. This is especially true of two of the samples. The reduced glutathione disappeared at approximately the same rate in all of the samples during the 4-hour incubation period. At the close of the 4-hour period the glutathione had decreased to values less than 2 mg. per cent. In fact, one sample showed complete loss of the glutathione at the close of the incubation period.

Addition of glutathione to heated milk: Glutathione was added at the rate of 50 mg. per cent to raw milk and to the same milk which had been heated momentarily to 70° C., 80° C., and 90° C. The results are shown in figure 2.

This figure illustrates the stabilization effect of the previous heat treatment of the milk on the glutathione. The raw milk shows rapid disappearance of the glutathione, similar to that shown in figure 1. Heating of the milk to 70° C. retarded, but did not prevent, the disappearance of the glutathione. However, when the milk had been subjected to temperatures of 80° C. or 90° C., it exhibited no destructive action on the glutathione. Actually, there was a slight increase in the glutathione values of these samples during

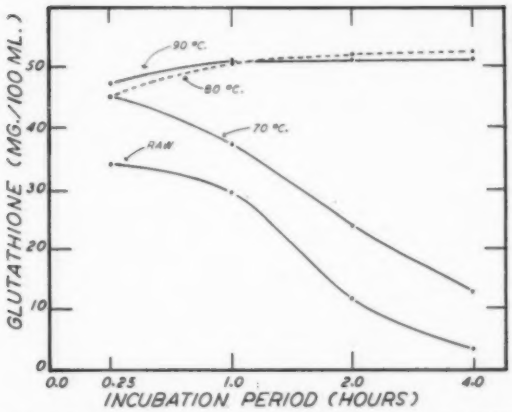

FIG. 2. The stabilization effect of heat on glutathione in milk (GSH added at rate of 50 mg. per cent).

the 4-hour period. This slight increase may be due to changes in the milk which have been brought about by the heat treatment.

Changes in the reduced and total glutathione content in milk and milk serum: The disappearance of reduced glutathione in milk raises a question; *i.e.*, whether the glutathione is merely changed to the oxidized form, or whether it is entirely destroyed. Steps were taken to determine which of these processes occurs.

Glutathione was added at the rate of 50 mg. per cent both to whole raw milk and to rennet serum prepared from the same milk. The reduced and

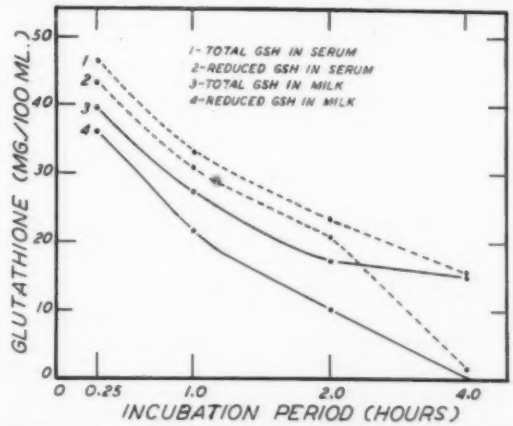

FIG. 3. The changes of reduced and total glutathione content in raw milk and in raw milk serum (GSH added at rate of 50 mg. per cent).

total glutathione content was determined after incubation periods as in the previous experiment. The total glutathione content was determined by reducing a portion of the sulfosalicylic acid filtrate with zinc dust according to the method of Woodward and Fry (3). Thus the difference between the reduced and total glutathione content represents the concentration of the oxidized form of glutathione. The results of this experiment are shown by figure 3.

The figure shows both the reduced and total glutathione values of the milk serum to be above those for the whole milk. The difference between the serum and milk values at the beginning of the incubation period is approximately 6–8 mg. of glutathione per 100 ml. This slightly higher value in the serum may be due partly to the dilution effect of the milk casein in the case of the whole milk or to a slight tendency of the casein to combine with the glutathione. However, the slight difference obtained between the two systems indicates that the casein *per se* plays no significant part in causing the disappearance of the glutathione.

Figure 3 shows further that the rate of disappearance of reduced glutathione is approximately as great in the serum as in the milk, although there was a slightly slower disappearance in the case of the serum.

The third important fact shown by the graph is that the major portion of the reduced glutathione which disappears in the milk is completely destroyed and is not merely changed to the oxidized form. The reduction of the acid filtrate by zinc dust at the various time intervals does increase the KIO_3 titer, indicating that a portion of the glutathione is in the oxidized form. However, the difference between the total and reduced glutathione after 0.25 hour and 1 hour in the case of the milk, and after 0.25 hour, 1 hour, and 2 hours in the case of the serum is relatively small, amounting roughly to about 2–4 mg. of glutathione per 100 ml. A much wider difference between the total and reduced glutathione values was secured at the 4-hour period, the difference amounting to about 15 mg. The total glutathione content appears to be leveling off at about this value at the close of the incubation period.

DISCUSSION

The actual cause for the destruction of glutathione when it is added to raw milk can only be speculated upon. The fact that temperatures of 80–90° C. stabilize the glutathione may be taken to indicate that the destruction is enzymic in nature. However, one should not be too prone to accept this as being the proper explanation, since these temperatures are sufficiently high to cause the formation of volatile sulfides, thus producing a reducing system which may be capable of preventing destruction of the glutathione. The temperatures which were effective in preventing the destruction, however, are high enough to destroy the principal oxidizing enzymes of milk.

The proteins of milk may be involved in the disappearance of the glutathione, although the casein is apparently of little significance in this connection. If the albumin and globulin of milk are concerned with the destruction of the tripeptide, the stabilization effect of heat may be due to changes in these proteins which occur, and which may, in turn, be correlated with sulfide liberation.

The results herein reported show that when 50 mg. of glutathione are added to 100 ml. of milk or milk serum the glutathione is practically gone at the end of the 4-hour incubation period. These changes were observed by using the nitroprusside test as well as by the quantitative method, although only the quantitative results are reported. In other trials in which 100 mg. of glutathione were used, considerable glutathione remained at the close of 4 hours at 20° C. This would indicate that the destroying agent in the milk is incapable of causing as complete destruction of this high concentration of glutathione in the 4-hour interval as it does with concentrations of 50 mg. per cent.

CONCLUSIONS

Raw milk or rennet serum from the milk causes a rapid disappearance of added reduced glutathione.

The major portion of the glutathione which disappears is destroyed, especially during storage periods up to 2 hours, since only a relatively small quantity was found to be in the oxidized form. After 4 hours of storage, however, a greater proportion of the missing glutathione is found in the oxidized form.

Heating milk momentarily to 80 or 90° C. and then cooling, prior to adding the glutathione, resulted in complete stabilization of glutathione.

A lower temperature of 70° C. was less effective in preventing its disappearance.

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PROTECTIVE INFLUENCE OF GLUTATHIONE ON COPPER-INDUCED OXIDATION OF ASCORBIC ACID IN MILK*

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The ability of glutathione to be easily oxidized and reduced has aroused much interest as to its function in those biological tissues and fluids where it is normally present. During the past few years, many valuable contributions have resulted from research pertaining to the role of glutathione in biological systems. Of especial interest, however, insofar as this paper is concerned, are those findings which indicate a possible close relationship between glutathione and ascorbic acid.

Hopkins and Morgan (6) found glutathione to protect ascorbic acid from oxidation by copper. This protective action was ascribed as being due to the fact that the glutathione "forms a stable compound with the copper, preventing efficient contact between the metal and its substrate."

Barron, Barron, and Klemperer (1) also found glutathione to efficiently inhibit copper catalysis of ascorbic acid and expressed the belief that this inhibition may be due to the strong affinity of glutathione for copper to form a copper-glutathione complex. These workers found practically complete inhibitory action by glutathione as long as there existed more than one molecule of glutathione per atom of copper.

Differences of opinion exist as to the ability of glutathione to prevent enzymic oxidation of ascorbic acid. Hopkins and Morgan (6) observed that glutathione protected ascorbic acid from oxidation by the enzyme "hexoxidase," but that the glutathione was itself oxidized. This protective action persisted until the glutathione had substantially disappeared from the system. Stotz, Harrer, and King (9) attribute the catalytic oxidation of ascorbic acid in certain plant juices to copper present in combination with protein material, rather than to a specific "oxidase," and found that a mixture of copper and albumin assumed the characteristic properties of these suggested enzymes. However, in later work these workers found guinea pig liver brei brought about slow aerobic oxidation of ascorbic acid, which they attributed to the "indophenol oxidase-cytochrome system." The glutathione naturally present in the liver brei was unable to protect added ascorbic acid from oxidation. This finding may indicate that glutathione is specific for copper catalysis of ascorbic acid, a view held by Barron, *et al* (1).

No studies have been reported on the inter-relationship of glutathione and ascorbic acid in milk, possibly because glutathione has never been shown

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to be a normal constituent of this product. It appeared desirable, however, in conjunction with other studies (4), to determine if added glutathione would protect ascorbic acid against copper-induced oxidation. Results of this study may help to explain the stabilization effect of high temperature heat treatment of milk on ascorbic acid, such treatment resulting in the formation of reducing compounds (5, 7). In addition, knowledge of the glutathione-ascorbic acid relationship in milk may be applied also to other biological systems.

EXPERIMENTAL PROCEDURE

Milk used for this experiment was obtained from individual cows of the Michigan State College herd. The milk was collected directly in procelain containers, cooled and placed in glass bottles. In order to study the protective action of glutathione, it was necessary to prevent the disappearance of the glutathione which occurs naturally in raw milk (4). This was accomplished by heating the milk in a glass flask to 80° C., a temperature which was previously found to be sufficiently high to inhibit this disappearance (4). Immediately after the heat treatment, the milk was cooled and divided into four lots. The lots were treated as follows:

Lot 1—Control.

Lot 2—1.5 p.p.m. of copper as copper sulfate.

Lot 3—1.5 p.p.m of copper plus either 25 or 15 mg. per cent of glutathione (Pfanstiehls).

Lot 4—Either 25 or 15 mg. per cent of glutathione.

The samples were stored at 4° C. and examined for ascorbic acid and glutathione after 15 min., 1 hr., 2 hrs., 4 hrs., 6 hrs., 24 hrs., and 48 hrs. The ascorbic acid determinations were made by titration with 2, 6-dichlorophenolindophenol according to the method of Sharp (8). The glutathione determinations were conducted by the macro KIO_3 titration procedure used previously (3) and adapted from the micro method of Woodward and Fry (11). Corrections were made for the potassium iodate titer of the control sample in order to arrive at the actual glutathione content of the samples in Lots 3 and 4.

RESULTS

The first portion of the experiment consisted of studying the influence of 25 mg. per cent of glutathione on stabilizing the ascorbic acid against copper catalysis. As stated in the procedure, the milk used in the trials had previously been heated to 80° C. to inhibit the disappearance of the added glutathione which occurs in normal raw milk. It is recognized that such heat treatment will tend to stabilize the ascorbic acid (2, 7). The results from individual trials were practically identical. Consequently, averages were secured and are graphically shown in figure 1.

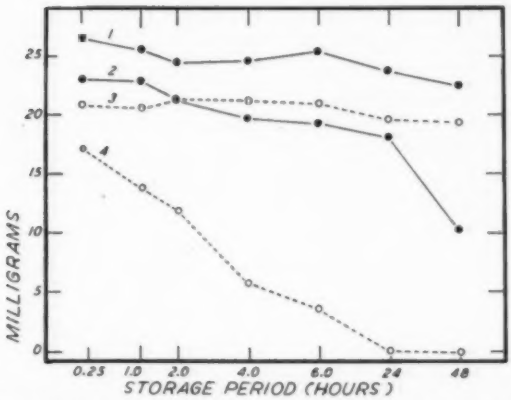

FIG. 1. The ability of 25 mg. per cent of glutathione to prevent copper-induced oxidation of ascorbic acid. (Ascorbic acid as mg. per liter, glutathione as mg. per 100 ml.).

Curve 1—Glutathione in sample containing no copper.

Curve 2—Glutathione in sample containing 1.5 p.p.m. of copper.

Curve 3—Ascorbic acid in sample containing glutathione and 1.5 p.p.m. of copper.

Curve 4—Ascorbic acid in sample containing 1.5 p.p.m. of copper but no glutathione.

This figure shows glutathione, in the concentration used, to greatly protect the ascorbic acid from copper catalysis throughout the 48-hour storage period. The ascorbic acid values remained substantially unchanged in the milk containing both copper and glutathione, whereas it had practically disappeared within six hours in the lot which contained copper but no glutathione. Although the ascorbic acid values for the control lot are not shown, they changed but slightly during the storage period, usually decreasing only 2 to 3 mg. per liter.

Figure 1 also shows the changes in the actual glutathione content in the lot which contained added copper and in that to which no copper was added. The GSH content of the milk which contained no added copper remained practically uniform throughout the period, the slight decrease which did occur amounting to approximately 4 mg. However, in the presence of copper the GSH slowly but definitely disappeared. The GSH value had dropped from approximately 23 mg. per cent to 18 mg. per cent during the first 24 hours, and had decreased still further to approximately 10 mg. per cent by the close of the 48-hour period. Considering that 25 mg. per cent of glutathione had been added to the milk, the results indicate that approximately 60 per cent of the reduced glutathione had disappeared.

Somewhat similar results were secured when 15 mg. per cent of glutathione were added to the milk. This is shown by figure 2. In these trials, however, the ascorbic acid was not as completely protected as in those in

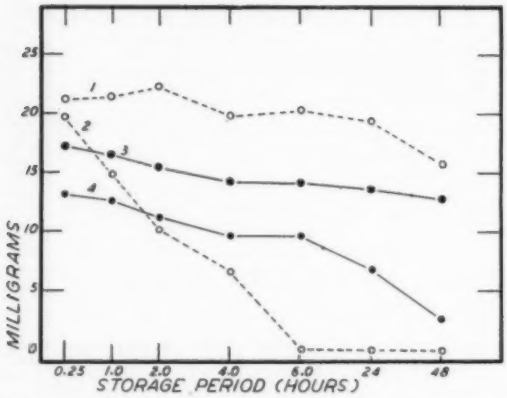

FIG. 2. The ability of 15 mg. per cent of glutathione to prevent copper-induced oxidation of ascorbic acid. (Ascorbic acid as mg. per liter, glutathione as mg. per 100 ml.).

Curve 1—Ascorbic acid in sample containing glutathione and 1.5 p.p.m. of copper.
Curve 2—Ascorbic acid in sample containing 1.5 p.p.m. of copper but no glutathione.
Curve 3—Glutathione in sample containing no copper.
Curve 4—Glutathione in sample containing 1.5 p.p.m. of copper.

which 25 mg. per cent of GSH were used. The ascorbic acid content of the milk containing 15 mg. per cent GSH plus copper showed slight decreases after 24 and 48 hours; the total decrease amounting to approximately 5 mg. In contrast to this, the milk containing copper but no glutathione had lost all of its ascorbic acid after 6 hours.

Figure 2 again shows disappearance of reduced glutathione in the milk containing added copper, the GSH content dropping from 23 mg. per cent after 15 minutes to approximately 3 mg. after 48 hours. Therefore, the total loss in GSH amounts to approximately 12 mg. per cent, or 80 per cent of the total which was added. The glutathione in the milk which contained no added copper showed a slight loss of approximately 2 mg. per cent.

In connection with the glutathione content of the milk to which no copper was added, it may be observed in both figures that the values after 15 minutes are slightly higher than would be expected on the basis of the 25 and 15 mg. per cent which were added. This slightly higher value has been observed many times, and likely results from changes which have occurred in the milk due to the heat treatment to which it was subjected.

DISCUSSION

The results secured in this study show that when glutathione is added to milk, previously heated to 80° C., it exhibits marked protective action against copper catalysis of ascorbic acid oxidation. The concentrations of gluta-

thione used in this study, *i.e.* 15 and 25 mg. per cent, were sufficient to almost entirely prevent oxidation of the ascorbic acid for the 48-hour period as induced by 1.5 p.p.m. of copper. The inhibiting power of 25 mg. per cent glutathione was sufficient to inhibit all oxidation of the ascorbic acid, whereas the 15 mg. per cent appeared to be somewhat less effective.

Although the ascorbic acid was not oxidized in the presence of the glutathione and copper, the glutathione itself was affected. This indicates that the glutathione is removed from the system by copper either by oxidation or combination. In the trials in which 15 mg. per cent of glutathione were added, the slight loss of ascorbic acid may have resulted due to the fact that the major portion of the glutathione had been destroyed, thus allowing the copper to oxidize the ascorbic acid. It is interesting to note that the proportion of glutathione to copper in these trials far exceeds the proportions of one molecule GSH: one atom copper which Barron, *et al* (1) found sufficient to prevent ascorbic acid oxidation.

CONCLUSIONS

The addition of 25 mg. per cent of glutathione to milk previously heated to 80° C. inhibited copper oxidation of ascorbic acid over a 48-hour period. Fifteen mg. per cent of glutathione was only slightly less efficient in preventing the catalytic action of copper.

Although the glutathione prevented copper catalysis of the oxidation of ascorbic acid, it slowly disappeared from the system.

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CHANGES OBSERVED IN MILK "SHAM FED" TO DAIRY CALVES[1]

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The specific digestive rôles played by saliva and esophageal secretions of ruminants are somewhat obscure. Since the saliva of the bovine presumably contains insignificant amounts of enzymes, the primary functions generally ascribed to the mixed secretions in the mouth are restricted to those of a physical nature. The importance of these secretions is readily recognized when the diet consists of solid foods but is probably under evaluated when the diet is liquid. Yet milk is listed among the food substances that produce the richest flow of saliva (1).

As is commonly observed, the young calf secretes copious amounts of saliva, particularly when nursing. It is reasonable to assume that the secretions of the oral cavity and esophageal passage serve an important rôle in the assimilation and utilization of milk by the animal. Espe (5) observed that ingested milk collected from an esophageal fistula increased markedly in viscosity, the maximum being reached about seven minutes after collecting and finally disappearing by the end of seven hours. He further suggested that this initial increase in viscosity aids in retaining the milk in the abomasum until rennet and/or acid coagulation is complete.

The foregoing observations and suggestions prompted an investigation designed to discover some of the changes produced in consumed milk prior to its entrance into the stomach of the dairy calf.

EXPERIMENTAL METHODS

Experimental Subjects. Four fistulated calves, described in table 1, were employed in this investigation. The three rumen fistulae were established according to procedures already described (12) except for one modification; that is, instead of waiting for the rumen wall to heal to the skin before excising the exposed area of the rumen wall, the opening was made immediately after the wall was sutured to the skin.

The gun-barrel esophagostomy operative techinque used was similar to that described by Espe (5). The calf was completely anaesthetized by intravenous injections of a chloral hydrate solution. Subsequently, the incision area, a region of the neck between 10 and 20 centimeters posterior to the angle of the jaw, directly over the jugular groove and slightly below the jugular vein was shaved, cleansed and disinfected. A five-centimeter longitudinal incision paralleling the esophagus was made through the

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TABLE 1

Experimental subjects and systems of milk feeding

Herd No.	Breed	Age	Type of fistula	Systems of feeding milk
		days		
C–55	Holstein	147	Ruminal	Alternating*: Open pail and nipple
A–117	Jersey	46	Ruminal	Alternating: Open pail and nipple
A–119	Jersey	43	Ruminal	Alternating: Open pail and nipple
42	Guernsey	22	Esophageal	Nipple only

* Each system was used one day, two feeds, before changing.

skin and underlying fascia. The muscles were separated, and the esophagus was freed from the surrounding tissue by blunt dissection, taking precaution to avoid breaking any of the blood vessels. The esophagus was severed; the two free ends were drawn to the surface and sutured around the circumference to the skin at the respective extremities of the incision, the terminations of which were rounded to fit the curvature of the esophagus. The edges of the skin incision between the ends of the esophagus, a distance of about two centimeters, were also sutured. The wound was dressed daily until healing resulted, at which time the stitches were removed.

A rubber tube, inserted into the exposed ends of the esophagus and held in place by a string attached to the wall of the tube and passing around the neck of the calf, served as a conduit for normal milk feeding.

Experimental Feeds. Pasteurized whole milk, superheated pasteurized whole milk, unpasteurized separated milk and reconstituted separated milk were fed in the various experiments. Pasteurized milk was heated at 63°–64° C. for 30 minutes, and the superheated milk was heated an additional five minutes at 80° C. in order to inactivate any lipase that might have survived pasteurization. The superheating and the cooling were done immediately before feeding. The reconstituted separated milk consisted of one pound of spray-dried milk in nine pounds of water.

All milk fed, except the reconstituted, was from a composite of the entire daily production of a herd of approximately 100 cows in various stages of lactation. The average test of the whole milk was 4.3 per cent fat and that of separated was 0.05 per cent fat. Whenever it was necessary to store the milk, it was held at a temperature of 35° F. In no case was the milk retained for experimental feeding longer than 10 hours after pasteurization or separation.

Immediately preceding feeding, a sample of the unconsumed milk, control, was taken for comparing with the consumed, "sham-fed." These samples, control and "sham-fed," were subjected to the same tests under standard conditions.

Procedures for Sham Feeding Milk. The calves having rumen fistulae were fed alternately from open pails on the floor and nipple calf feeders* in an elevated position; whereas the esophagostomized calf was fed by the latter system only. The milk was fed at a temperature of 100° F. twice daily, the quantity at each feed amounting to approximately seven per cent of the body weight. The time required to ingest a given weight of milk by either system was determined by means of a stop watch.

FIG. 1. Method of "sham feeding" milk *via* cardial end of esophagus (rumin fistulated calf).

As the milk was consumed, it was withdrawn from either the cardial end (figure 1) or the medial section (figure 2) of the esophagus. In the first procedure, a glass tube having a rubber conduit attached was inserted approximately two inches into the posterior extremity of the esophagus, the entrance being made progressively *via* fistula, ruminal cavity and cardia. In order to prevent expulsion of the tube during deglutition, the conduit was held in place by hand. Thus a continuous passage was formed permitting the milk to flow from the mouth through the esophagus and conduit into an exterior receptacle.

As a means of ascertaining whether or not the point of the esophageal outlet was a factor to be considered, the milk was withdrawn by the second method (figure 2). During the collection period, the conduit was in the posterior end of the pharyngeal section of the esophagus.

* Coyner feeder pail, sold by the Armour & Company, U. S. Yards, Chicago, Ill.

FIG. 2. Method of "sham feeding" milk *via* medial section of esophagus (esophagostomized calf).

In all collections from the conduit the first two or three swallows of milk, which contained much accumulated saliva and some residues of solid feeds, were discarded. The remainder of the material was received as shown in figures 1 and 2 and subsequently examined.

Technique employed in examination of milk. Representative samples of control and consumed milks were subjected within 10 minutes after collection to the following examinations in the order listed: (a) hydrogen-ion concentration, (b) rennet coagulation, (c) curd tension, (d) lipolytic activity and (e) gravity creaming. In several cases, samples of milk were retained at a constant temperature of 38° C. for two hours, after which several of the tests were repeated.

A. Hydrogen-ion concentration. This was measured with a Beckman, glass electrode pH meter. Since the pH of the consumed whole milk changed rapidly, this determination was made within two minutes after collection.

B. Rennet coagulation. Except for the modification of the temperature at which the milk was held, the procedures employed in this determination were as outlined by Sommer and Matsen (11). Since the body temperature of cattle is approximately 38° C., this degree of heat was considered preferable, from a physiological point, to the prescribed 30° C. for tempering the milk.

C. Curd tension. The methods used for measuring curd strength were the same as recommended by the committee on Methods of Determining Curd

Tension of Milk (2) except that the milk was adjusted to a temperature of 38° C. instead of 35° C. The measuring apparatus used was the Submarine Signal Curd Tension Meter.

D. Lipolytic activity. The activity of lipolytic enzymes in the various samples of milk was estimated according to the general procedure of Nair (9). Five mls. of milk were added to 100 mls. of a sterilized sucrose-cream substrate. This was incubated at 37° C. for 21 days, during which period determinations of the increases in titratable acidity were made at various intervals. Since the rate of increase generally was retarded after six to eight days, the lipolytic data recorded in the tables represent the measurements on the seventh day. The lipolytic activity was expressed in terms of mls. of 0.1N · NaOH required to bring 10 gms. of the substrate admixture to the neutral point as indicated by phenolphthalein.

E. Gravity creaming. The cream volume, or layer, was used as an index of the relative stability of the emulsions of various milk samples. The extent of the fat rising was estimated on samples placed into 100 ml. graduated cylinders and held at room temperature for 24 hours, after which the depth of the so-called cream layer was measured.

EXPERIMENTAL RESULTS

The experimental observations involved several variables: pre-feeding treatment of milk, individual calves, and systems of "sham feeding," which were varied by the method of administration and by the point of collection from the esophagus. The data as presented in tables 2 to 5, inclusive, suggest that several of these variables were insignificant factors; whereas others were of primary importance.

Relation of feeding system to the changes produced in the milk. When similar systems of feeding were employed, the trend of changes noted in whole milk was essentially the same when collections were made from the cardial end of the esophagus (table 2) as when made from the medial region (table 3). These results indicated that if the secretions from any one section of the esophagus contribute to the alterations of the milk, the pharyngeal end is the primary region involved.

The consumed milk, irrespective of method of feeding, exhibited several properties markedly different from those of the control. A comparison of the magnitude of the alterations resulting from the two systems of feeding indicated that in whole milk the changes of all observed properties were more exaggerated when fed by the nipple system than from open pail (tables 2 and 4), but in separated milk there was no marked difference except in lipolytic activity (table 5).

Individual calves as a factor in the alterations of milk. The data in tables 2, 4 and 5 reveal considerable variation in the magnitude of the changes in milk "sham fed" to different individuals. The modification of

TABLE 2

Comparative changes observed in "sham fed" pasteurized whole milk subjected to various tests immediately after collection from the cardial end of the esophagus

Method of feeding	Herd No. of calf	Observations	Rate of cons.	Rennet coag. time	Curd tension		pH	Lipolytic activity	Cream volume
					Surface	Body			
			lbs./min.	*min.*	*gms.*	*gms.*		*mls. NaOH*	*per cent*
Control	.....	10	.....	10.53	40	38	6.39	0.53	8.8
Open pail	C–55	5	16.6	6.35	47	41	6.32	1.97	32.7
	A–117	5	3.8	3.04	45	39	6.01	4.33	69.8
	A–119	5	2.1	4.36	44	39	6.22	2.35	45.3
	Average	...	7.5	4.58	45	40	6.18	2.88	49.3
Nipple pail	C–55	5	2.3	3.26	49	45	6.00	3.50	69.8
	A–117	5	1.6	1.95	50	47	5.93	4.45	98.0
	A–119	5	0.9	1.93	47	41	5.81	4.95	89.5
	Average	...	1.6	2.38	49	44	5.91	4.30	85.8

TABLE 3

Comparative changes observed in various samples of milk "sham fed" via esophageal fistula and tested immediately after collection

Kind of milk	Treatment of milk	No. of observ.	Rennet coag. time	Curd tension		pH	Lipolytic activity	Cream volume
				Surface	Body			
			min.	*gms.*	*gms.*		*mls. NaOH*	*per cent*
Pasteurized whole	Control	2	11.20	40	35	6.30	0.90	9.0
	Consumed	2	3.63	43	43	5.70	5.25	99.0
Reconstituted skim	Control	1	17.51	7	7	6.18	0.60	.....
	Consumed	1	16.80	11	11	6.20	5.95	.....

TABLE 4

Comparative changes observed in "sham fed" superheated whole milk subjected to various tests immediately after collection from the cardial end of the esophagus

Method of feeding	No. of expt. subj.	No. of observ.	Milk fed	Rate of cons.	Rennet coag. time	Curd tension		pH	Lipolytic activity
						Surface	Body		
				lbs./min.	*min.*	*gms.*	*gms.*		*mls. NaOH*
Controls	.....	2	A	.....	20.98	19	19	6.37	0.63
	.....	1	B	.....	19.96	25	25	6.48	0.50
Open pail	C–55	1	B	16.5	19.39	26	26	6.44	2.50
	A–117	2	A	4.4	15.06	23	22	6.18	5.47
	A–119	1	B	3.6	19.11	28	28	6.42	2.65
	Average difference from controls*			.....	– 3.31	+3	+3	–0.11	+3.45
Nipple pail	C–55	2	A	3.3	17.87	27	25	6.20	4.73
	A–117	1	B	2.6	7.58	25	25	6.49	4.85
	A–119	2	A	1.4	15.51	25	25	6.17	4.67
	Average difference from controls*			.....	– 5.91	+6	+5	–0.18	+4.13

* Since contols varied considerably, the average for "sham-fed" milks is expressed in terms of difference from their respective controls.

TABLE 5

Comparative changes observed in "sham fed" unpasteurized separated milk subjected to various tests immediately after collection from the cardial end of the esophagus

Method of feeding	Herd No. of calf	Observations	Rate of cons.	Rennet coag. time	Curd tension		pH	Lipolytic activity
					Surface	Body		
			lbs./min.	*min.*	*gms.*	*gms.*		*mls. NaOH*
Control	.....	4	.....	10.89	64	61	6.48	1.29
Open pail	C–55	2	22.7	10.03	67	58	6.38	2.97
	A–117	2	4.3	9.80	57	48	6.55	5.67
	A–119	2	4.0	9.76	56	49	6.43	3.05
	Average	...	10.3	9.86	60	52	6.45	3.90
Nipple pail	C–55	2	3.0	10.61	65	56	6.55	4.55
	A–117	2	2.3	8.98	58	43	6.37	5.55
	A–119	2	1.7	10.16	54	46	6.52	5.65
	Average	...	2.3	9.92	59	48	6.48	5.25

the milk was less pronounced in the case of C–55, an older calf, than in the case of either A–117 or A–119. Though these last two were about the same age, there was a marked difference in vigor. E–219 was somewhat unthrifty and apparently secreted less saliva. Closely associated with the degree of change in the milk consumed by different calves were the rates of consumption. Whether or not this relationship is an *a priori* condition remains to be determined. Since the system of feeding each calf was alternated from day to day, the variations attributable to this factor are compensatory and consequently do not play an important rôle in the average.

Effect of pre-feeding treatment of milk on the changes resulting from sham feeding. When pasteurized whole milk was "sham fed," the properties of the milk were markedly altered as indicated in tables 2 and 3. These changes involved a pronounced reduction in rennet coagulation time, a slight increase in curd tension, a striking decrease in pH, an increase in cream volume and an augmentation of lipase activity. The trend of the changes for "sham-fed" superheated whole milk (table 4) were essentially the same as for the pasteurized, but the extent of the modification was somewhat less, except in lipolytic activity. In the case of unpasteurized separated milk (table 5) there was a slight reduction in rennet coagulation time, a tendency to diminish the strength of the body of the curd, a rise in lipolytic activity but no significant change in pH. "Sham feeding" reconstituted skim milk (table 3) produced few definite changes other than an increase in lipolytic activity. Since the number of determinations was exceedingly limited, no specific significance can be ascribed to the apparent increase in curd tension, which in the control was relatively low.

In accordance with reports of other investigators, a comparison of the various samples indicates that superheating greatly increased the rennet coagulation time (10, p. 230) and reduced curd tension (2); whereas removal of fat apparently strengthened the curd tension, surface and body, but did not affect perceptibly the rate of rennet coagulation.

Changes in the properties of various samples of milk retained two hours. In order to determine the comparative changes in the milk over a period of time, the various control and "sham-fed" samples were retained at 38° C. for an arbitrary period of two hours. After this time, the tests were repeated. A comparison of the changes observed immediately after "sham feeding" with those made two hours later is presented in table 6. In the case of the pasteurized whole milk little change was noted in the control, but in the "sham-fed" there was a continued reduction in the rennet coagulation time, a diminution in the curd tension, particularly in the case of the nipple system of feeding, and a further lowering of the pH. Except for a slight decline in the curd tension of the control sample, the changes in the superheated milk were essentially the same as for the pasteurized. In contrast with whole milks, the only conspicuous modification of the control separated

TABLE 6

Relative changes observed in various samples of control and "sham fed" milks retained at 38° C. for a period of two hours

Kind of milk	System of feeding	Time of observ.	No. of observ.	Rennet coagulation time	Curd tension		pH
					Surface	Body	
				min.	*gms.*	*gms.*	
	Control	Immed.	5	10.52	40	39	6.35
		2 hrs.	5	11.18	38	37	6.51
Pasteurized whole milk	Open pail	Immed.	7	4.31	48	41	6.10
		2 hrs.	7	1.74	42	41	5.82
	Nipple pail	Immed.	8	2.35	49	44	5.90
		2 hrs.	8	1.24	31	31	5.56
	Control	Immed.	2	18.92	24	24	6.42
		2 hrs.	2	20.99	18	18	6.53
Superheated whole milk	Open pail	Immed.	3	17.38	27	27	6.34
		2 hrs.	3	9.94	21	21	6.17
	Nipple pail	Immed.	3	12.11	31	30	6.30
		2 hrs.	3	1.13	18	18	5.59
	Control	Immed.	2	11.23	65	63	6.54
		2 hrs.	2	10.51	57	53	6.53
Unpasteurized skimmilk	Open pail	Immed.	3	10.27	59	53	6.45
		2 hrs.	3	8.40	69	54	6.44
	Nipple pail	Immed.	3	10.36	59	48	6.55
		2 hrs.	3	8.19	69	53	6.48

milk was a decreased curd tension; whereas in the "sham-fed" sample there was a slight rise in curd strength and an acceleration in rate of rennet coagulation but no change in pH.

From general appearance the viscosity of all milks was augmented by "sham feeding." This reaction seemed to be more evident in whole milks, particularly the pasteurized, than in the separated. The viscosity of "sham-fed" milk increased rapidly, reaching the maximum within ten minutes, but abated slowly as evidenced by its persistence at the end of the two hours holding period.

DISCUSSION

In view of reported information relative to the properties of bovine saliva, it was logically assumed that its combination with whole milk by "sham feeding" would result in a mixture having a pH somewhere between the values for the two fluids, pH 8.1 for saliva and 6.6 for milk. Preliminary observations revealing a reduction of pH below that of the control milk were rather baffling; but subsequent experiments helped to clarify these paradoxical results. A pronounced rancidity accompanying the increased hydrogen-ion concentration suggested that lipolysis was involved, which indication was substantiated in subsequent tests. A comparison of the hydrogen-ion

concentration of "sham-fed" whole milk with that of "sham-fed" separated milk indicated that fat was a constituent essential for the change in reaction. Thus the decreased pH of the "sham-fed" whole milk becomes explainable on the basis of the liberation of fatty acids from the milk fat by lipolysis.

These results focused attention on the problem of ascertaining the origin of the lipolytic agents. Since saliva is generally considered to be devoid of lipase, attention was directed toward other sources. Inasmuch as milk frequently contains lipase (10, p. 30), the possibility that the increased lipolysis in "sham-fed" milk might have been due to the activation of enzymes present in the control milk was considered first. This conjecture was invalidated by the discovery that "sham-fed" milk previously superheated to insure inactivation of lipase manifested as much lipolytic activity as the "sham-fed" pasteurized milk.

Thus it appears that the lipolytic agents are in the fluids that are mixed with the milk in the process of consumption. Their origin evidently is either in micro-organisms present or in various secretory glands. Preliminary bacterial investigations of control and "sham-fed" milks showed a marked increase in total numbers of bacteria in the latter. This increase included some lipolytic organisms, which were hardly adequate to account for the pronounced lipolysis of the "sham-fed" whole milk. This suggestive evidence merits further investigation.

As to the glandular origin, the possibility that the lipolytic substances are extracellular enzymes cannot be excluded. Koebner (6) detected lipase in the saliva of sheep and oxen. Koldayev and Pikul (7) found that saliva from the parotid and submaxillary glands of dogs has definite lipolytic action. These reported observations in conjunction with the foregoing results are provisional evidence for postulating that the lipolytic activity detected in the "sham-fed" milks may be due to secreted enzymes. In addition to the probable involvement of the salivary glands, the glands in the pharyngeal end of the esophagus (4, p. 239), the function of which has not been established, are possibly also implicated.

The extent to which the various modifications of the "sham-fed" milks are attributable directly to lipolysis and the accompanying change in pH is not definite. The complex physical and chemical nature of the saliva and associated fluids no doubt influences the character of the milk in many ways. From a physical standpoint dilution is one of the direct effects.

As reflected in curd strength measurements, simple dilutions decrease the tension (2). The slight initial decrease of this value in the body of "sham-fed" separated milk was probably a manifestation of the dilution phenomenon, which in whole milk was evidently counteracted by increased acidity. This reaction according to Doan (2) toughens the curd as the pH drops from the normal (6.4 to 6.7) to approximately 5.9 but decreases curd tension from this point down to the isoelectric point (pH 4.7). This phe-

nomenon is best exemplified by the changes observed in whole milk "sham fed" from a nipple pail (table 6).

Before "sham feeding," the pH was 6.35 and curd tension for surface and body, respectively, was 40 grams and 39 grams. Immediately after feeding, the respective values were 5.90, 49 and 44, but two hours later the corresponding readings were 5.56, 31 and 31. Throughout, the changes in curd tension are small but relatively consistent, which contributes to their possible significance. Though lipolysis and dilution evidently are important factors affecting the curd tension of "sham-fed" milks, the changes probably should not be attributed to these two factors alone.

The retarding effect of dilution on rate of rennet coagulation was counteracted, in part, by increased acidity. As the pH decreased, there was an acceleration in the rate of coagulation, which observation is in accord with the results of other investigators (10, p. 232). That the increased acidity was not the only accelerating factor is suggested by the slight reduction in the coagulation time of "sham-fed" separated milk, the pH of which was not changed perceptibly.

Another characteristic of the "sham-fed" milks was their comparative stability as manifested by the slight fat rising in whole milk and by the prolonged time necessary for wheying to start in both whole and separated milks. These properties probably are associated with the increased viscosity, a phenomenon also observed by Espe (5). Perhaps one of the primary constituents contributing to the viscosity and tending to maintain a uniform distribution of fat in the milk is the mucin of the saliva.

The quantity of saliva secreted, the extent to which it is mixed with the milk and probably the manner of mixing are factors affecting the degree of modification of "sham-fed" milks, particularly whole milk. The more copious the salivary flow and the more the secretions are mixed with the milk, the greater are the alterations. The first several swallows of milk come in contact with more saliva and other secretions than the remainder, due primarily to the pre-feeding salivary flow, a response to psychic stimuli. The constancy of the salivary flow after milk feeding is initiated remains an unsolved problem.

The amount of salivary secretion apparently varies with the age, with the manner of milk consumption and with the vigor of the individual calf. As the calves grow older, the rate of milk consumption accelerates without an apparent commensurate increase in salivary flow, thus resulting in less saliva per unit of milk ingested. The nipple system of feeding in contrast with the open pail method stimulates salivation and retards the rate of consumption, resulting not only in more saliva per unit of milk but also in more mixing. In addition it is possible that the agitation to which the milk is subjected during nursing promotes lipolysis (8). Suppression of saliva secretion apparently is associated with an unthrifty state of the individual, but *a priori* relationships have not been established.

According to Dukes (4, p. 234) the character of the food affects not only quantity but also quality of the saliva. It is possible that the milk excites secretions from glands that are not generally stimulated by other foods. Furthermore, the qualitative response of the salivary glands to any one dietary constituent perhaps is governed to some extent by the age of the animal. Therefore, if the lipolytic activity in "sham-fed" milks is due to a secretory product, it is possible that this reaction is restricted to young calves consuming milk.

It is difficult to assess the true significance of the foregoing *in vitro* observations of "sham-fed" milks in terms of specific *in vivo* reactions. However, it appears that digestion of milk fat is initiated prior to its passage into the stomach of the calf. The accompanying changes, including increased viscosity, probably aid in accelerating rennet coagulation in the abomasum, which reaction is in accord with Espe's theory (5). That is, quick and thorough coagulation is desirable to prevent flooding of the small intestine, which organ is very sensitive. The slightly increased curd tension observed does not necessarily adversely affect digestibility of the coagulum (3) as previously suspected. Seemingly, then, the initial ingestive changes in milk, whole milk particularly, facilitate its subsequent digestion.

The nipple feeding in comparison with the open pail system augments the degree to which "sham-fed" milk is modified. Nursing, in addition to aiding in the direct passage of milk to the abomasum (12) evidently promotes initial changes in the character of milk expediting subsequent digestion and utilization. The advantages of the nipple system in pre-gastric changes of milks apparently are not as striking in separated milk as in whole. However, this must not be considered as an imputation against the practice of feeding skimmilk by the nipple method. In the final analysis the foregoing fundamental observations increase credence in the value of the nursing system for feeding milk to young calves.

SUMMARY

1. Pasteurized and superheated whole milks and separated milk were "sham fed" either from open pails or through nipples to fistulated calves.

2. A comparison of the properties of various samples of "sham-fed" milks with properties of their respective controls revealed in the case of pasteurized whole milk increases in rate of rennet coagulation, in tension of the body of the curd, in hydrogen-ion concentration, in lipolytic activity and in cream volume. The corresponding changes in superheated whole milk were in the same direction but of less magnitude. Separated milk manifested a slight increase in the rate of coagulation, an insignificant decrease in curd tension, no pronounced change in hydrogen-ion concentration but a marked increase in lipolytic activity.

3. Further alterations were evident after retention of the samples at 38° C. for two hours. Except for a slight increase in pH there were no marked changes in the control sample of pasteurized whole milk, but in the "sham-fed" samples all initial changes, except for a reduction in curd tension, were further amplified. The trends of all samples of superheated whole milk were in the same direction as in pasteurized whole milk. In the separated milk the only notable change in the control sample was a decrease in curd tension, whereas in the "sham-fed" sample there was a slight increase in rate of coagulation and in curd tension.

4. Several of the pronounced changes in the properties of the whole milk as contrasted to those of the separated milk were attributed in part to lipolysis, a reaction probably resulting from enzymes present in saliva and/or other secretions of the mouth and esophagus.

5. Ingestion of milk through a nipple as compared with consumption from an open pail exaggerated the changes in whole milks but produced little difference, except in increased lipolytic activity, in separated milk.

6. The pre-gastric alterations of milk apparently are of such a nature as to expedite subsequent digestion and assimilation.

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THE EFFECT OF PHOSPHORIC ACID SILAGE ON THE ACID–BASE BALANCE IN DAIRY COWS

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The use of mineral acids for the preservation of ensiled crops has raised the question of their physiological effects, particularly upon acid-base balance. There are several reports in the literature, dealing mostly with A.I.V. silage which contains hydrochloric acid or a mixture of hydrochloric and sulfuric acids. Hayden and co-workers (4) fed A.I.V. silage to dairy cattle as the only roughage for five months. A nearly complete disappearance of bicarbonates in the urine resulted while ammonia excretion increased as much as 40-fold. The CO_2 combining capacity of the blood plasma was slightly reduced. These effects were not cumulative and did not reach a dangerous point. Peterson and coworkers (8) and Bohstedt and coworkers (1) report similar changes in blood and urine values when feeding A.I.V. silage as the only roughage. The silage was supplemented with four ounces of limestone daily in one experiment and, in the second, with one ounce of a mixture of 10 parts of $CaCO_3$ to 3 parts of Na_2CO_3 per fifteen pounds of silage. Changes occurring in the acid-base balance were considered to be of little significance, or at least not dangerous to the health of the animals.

Crasemann (3) likewise reports urinary acidosis and a reduced alkaline reserve of the blood plasma when feeding A.I.V. silage to dairy cows. He recommends supplementing the silage either with hay high in mineral elements or with $CaCO_3$ or $NaHCO_3$. He remarks that $CaCO_3$ does not always produce the desired results, and that the addition of basic salts is justified even when feeding hay as the latter does not always contain a sufficient basic surplus. Brouwer and Dijkstra (2) report the same findings when feeding unneutralized A.I.V. silage. The extent of the physiological changes was much diminished when a neutralizing agent was added to the silage. Virtanen (12) fed several cows during a winter period with rations containing large amounts of silage preserved with hydrochloric acid. No disturbances were detected other than a change of the urine pH from the alkaline to the acid side and a great decrease in the amount of combined CO_2 excreted. When soda or a mixture of soda and limestone was included in the ration, the reaction of the urine remained close to normal.

Mollgaard and Thorbek (5) carried out energy metabolism studies on seven cows fed A.I.V. silage. They report that the acidosis developed by the addition of mineral acids to silage resulted in a negative calcium balance and increased enormously the heat production. This increased heat

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production was sufficient to decrease significantly the nutritive value of the fodder. The further statement is made that neutralization with basic salts restored a positive calcium balance and decreased the heat production, but that the digestibility of the ration was significantly depressed, especially when sodium bicarbonate was used. Supplementing the ration with beet roots, however, had a very beneficial effect both from the standpoint of digestibility and content of net energy. Schnepf (9, 10) fed silage preserved with mineral acids to sheep. He reported that the silage caused a negative calcium and phosphorus balance and that the addition of dry hay or of a mineral supplement had a favorable effect although a positive balance was not restored in every case.

These reports demonstrate the desirability of neutralizing A.I.V. grass silage by the addition to the ration of either basic salts or roughages with a high basic surplus. The present paper deals with the effect of feeding phosphoric acid grass silage on the acid-base balance of dairy cows and represents a companion study to one dealing with the chemical changes in this type of silage (Pagé and Maynard, 6). The fact that phosphoric acid is normally concerned in the acid-base regulation of the organism and that phosphorus is an essential mineral, suggested that this acid might cause lesser physiological disturbances than the stronger acids used in preserving silage by the A.I.V. process. Three separate experiments are here reported.

EXPERIMENT I

Four animals well along in lactation were selected for this first study. During a four week preliminary period, the cows were fed corn silage with mixed hay and concentrates. The concentrate mixture contained 18 per cent of protein and included one per cent of bone meal. The mixed hay was composed of timothy, red top, quack grass, alfalfa, blue grass and red clover. The corn silage was from a crop that was well eared and at a medium stage of maturity. Following the preliminary period, the cows were placed for six weeks on a ration of phosphoric acid silage and grain. This silage was preserved with 27 pounds of 68 per cent phosphoric acid (food grade) per ton of green material. It had the following composition: grasses, 50 per cent; clovers, 25 per cent; alfalfa, 20 per cent; and weeds, 5 per cent.

During the preliminary period, blood was collected for analysis at the end of the 2nd, 3rd and 4th weeks, respectively. Twenty-four hour urine collections were made at the end of the 2nd and 3rd weeks. Five blood and urine collections were made during the period of acid silage feeding, namely, at the end of the 1st, 2nd, 3rd, 4th and 6th weeks.

The CO_2 combining capacity of the blood plasma was determined with a Van Slyke manometric blood gas apparatus (7). The pH of the urine was measured with the quinhydrone electrode, the fixed CO_2 with the Van Slyke

manometric blood gas apparatus and the ammonia nitrogen by the Folin aeration method as modified by Steele (11).

The results of these analyses for Experiment I are summarized in table 1. Clearly, there was no change in the pH of the urine as a result of feeding the phosphoric acid silage. While the fixed CO_2 in the urine was lower in every case during the acid silage period, the values during the latter period did not reach a sufficiently low level to indicate any tendency to acidosis. Clearly, the values for NH_3-nitrogen do not furnish any evidence of acidosis since they were actually lower during the silage period. It is also clear that the blood CO_2 combining capacity was not significantly altered. Thus all of the data in table 1 are in agreement in indicating that there was no tendency to acidosis when the phosphoric acid silage was fed. It should finally be stated that no detrimental effect followed the period of acid silage feeding with respect to weight, general appearance and milk production of the animals.

EXPERIMENT II

Four cows in heavy milk production were selected for this experiment. They were fed a ration containing phosphoric acid grass silage, mixed hay and grain during one period of six weeks and a ration containing molasses silage, mixed hay and grain during another period of similar length. The grain mixture, mixed hay and acid silage were of the same composition as in the first experiment. The molasses silage was slightly higher in legumes than the acid silage, its approximate composition being as follows: grasses, 35 per cent; clovers, 40 per cent; alfalfa, 20 per cent; and weeds, 5 per cent.

Urine was collected over a twenty-four hour period at the end of the 2nd and 6th weeks of the first period and at the end of the 1st, 2nd, 4th and 6th weeks of the second period. Blood samples were taken for the 3rd, 4th and 6th weeks of the first period and for the 1st, 2nd, 4th and 6th weeks of the second period. The analytical results of this experiment are summarized in table 2. These data clearly show that the pH of the urine was not changed as a result of feeding phosphoric acid silage. The changes which occurred in the fixed CO_2 and NH_3-Nitrogen of the urine are not consistent nor significant enough to indicate a condition of acidosis. Likewise, the alkaline reserves of the blood plasma show no significant changes from one period to the other. It may be concluded that no consistent evidence is presented in table 2 indicating that the feeding of phosphoric acid silage resulted in any tendency to acidosis, compared to the feeding of molasses silage.

EXPERIMENT III

In this last experiment, the phosphoric acid grass silage was submitted to a somewhat more crucial test in that the crop thus preserved contained only traces of legumes and the grain mixture fed with it in two of the four cases contained no neutralizing agent.

TABLE 1

Mean urine and blood values, Experiment I

Cow	Urine values						Blood plasma CO_2 combining capacity, volume %	
	pH		Fixed CO_2, mM (24 hours)		NH_3–Nitrogen, mg. (24 hours)			
	Prelim. period	H_3PO_4 period	Prelim. period	H_3PO_4 period	Prelim. period	H_3PO_4 period	Prelim. period	H_3PO_4 period
1	8.3	8.3	1607	762	323	146	69.6	66.7
2	8.5	8.2	2207	814	303	269	64.5	61.1
3	8.4	8.2	1792	505	209	90	65.1	66.2
4	8.3	8.2	1691	854	281	149	74.2	68.6

TABLE 2

Mean urine and blood values, Experiment II

Cow	Urine values						Blood plasma CO_2 combining capacity, volume %	
	pH		Fixed CO_2, mM (24 hours)		NH_3–Nitrogen, mg. (24 hours)			
	Molasses silage period	H_3PO_4 silage period	Molasses silage period	H_3PO_4 silage period	Molasses silage period	H_3PO_4 silage period	Molasses silage period	H_3PO_4 silage period
1	8.3	8.1	449	257	236	608	66.9	64.6
2	8.3	8.3	994	874	606	1547	72.9	67.6
3	7.9	8.2	215	538	122	207	61.5	67.2
4	8.3	8.3	1354	1542	439	327	67.5	72.8

Four cows, either dry or well along in lactation, were selected for this study. These animals were sterile but in thrifty condition. All experimental animals received corn silage and mixed hay for four weeks. The corn silage was from a well eared crop at a medium stage of maturity. The mixed hay, which was early cut and well cured, contained about 50 per cent of legumes. During this preliminary period, twenty-four hour urine collections were made at the end of the 2nd and 4th weeks. Blood samples were taken at the end of the 3rd and 4th weeks.

The cows were then placed on phosphoric acid silage alone for the next four weeks. This silage was made from a timothy crop, early bloom, which contained a small amount of other grasses and only a trace of legumes. It was preserved with 20 pounds of 68 per cent phosphoric acid (food grade) per ton of green material. At the end of this time, two of the animals received some grain in addition to the silage during the next four weeks, while the other two remained on phosphoric acid silage as the only feed. During this period, with the exceptions noted later, urine collections were made at the end of the 2nd, 7th and 9th weeks. Blood samples were taken at the end of the 2nd, 4th and 7th weeks.

During a further period of five weeks, the acid silage was neutralized as follows: nine ounces of ground limestone per 100 pounds of silage were given to one of the cows which had been receiving silage only, and also to one of the cows on the silage and grain ration. The other two cows received mixed hay in addition to their previous ration: cow No. 2 received an average of 5 pounds of hay daily and cow No. 4 received 4.5 pounds. The mixed hay was early cut and well cured. It contained clover, alfalfa, timothy and other grasses. The legumes constituted about fifty per cent of the crop. Urine and blood samples were taken after the cows had been on the modified rations for two weeks and again at the end of the fifth week.

When the cows were put on phosphoric acid grass silage as the only feed, three of the milking animals dried up quickly. Two of the four animals lost flesh and failed to eat properly. Only one cow (No. 4) remained in good condition throughout the experiment. All showed some improvement when either mixed hay or limestone was added to their diet. Cow No. 4 showed an increase in appetite, in that she cleaned up her feed more readily.

The results of the blood and urine analyses are summarized in table 3. The samples collected during the period of acid silage feeding were not entirely satisfactory. One of the cows (No. 1) went off feed during this period and was put on a ration containing a variety of feeds for several weeks. Table 3 includes only her last set of values in this period, obtained after she had been back on acid grass silage and grain for two weeks. One set of values for cow No. 4, obtained during the 7th week had to be discarded because it was found that she had been stealing hay from a neighboring animal. This point will be taken up later. In the case of animal No. 2, only

TABLE 3

Mean urine and blood values, Experiment III

Cow	Urine values									Blood plasma CO_2 combining capacity, volume %		
	pH			Fixed CO_2, mM (24 hours)			NH_3–Nitrogen, mg. (24 hours)					
	Period 1 Herd ration	Period 2 H_3PO_4 silage	Period 3 H_3PO_4 silage plus lime or hay	Period 1 Herd ration	Period 2 H_3PO_4 silage	Period 3 H_3PO_4 silage plus lime or hay	Period 1 Herd ration	Period 2 H_3PO_4 silage	Period 3 H_3PO_4 silage plus lime or hay	Period 1 Herd ration	Period 2 H_3PO_4 silage	Period 3 H_3PO_4 silage plus lime or hay
1	8.5	5.9	7.8[a]	1708	8	889[a]	243	2279	276[a]	60.5	.....	61.0[a]
2	8.1	5.7	8.3[b]	1099	3	490[b]	199	441	206[b]	57.5	59.0	.....
3	8.4	5.6	8.6[a]	1040	2	1093[a]	195	2748	265[a]	61.6	63.0	66.5[a]
4	8.5	5.5	8.3[b]	740	1	568[b]	401	718	197[b]	55.8	61.0	61.0[b]

[a] Silage plus limestone.
[b] Silage plus hay.

three of the seven blood samples could be analyzed because the other four hemolyzed on centrifugation. Her blood appeared to be abnormally high in cell volume. Unfortunately, it was impossible at the time to investigate this condition further.

In spite of these shortcomings, a rather clear picture is presented in table 3. It is noted that when the cows were changed from the herd ration to the phosphoric acid grass silage alone, there was a marked drop in the pH of the urine, a nearly total disappearance of the fixed CO_2, and a very marked rise in the urinary ammonia. These changes were not modified by the addition of grain which was made in the case of two of the cows during the last five weeks of period 2. The urinary values for period 3 show that when the phosphoric acid grass silage was supplemented by either limestone or hay, in the amounts previously stated, the tendency to acidosis was entirely overcome. The values for all urinary constituents became similar to those obtained during period 1.

An interesting bit of evidence relative to the effectiveness of hay in correcting the acid condition was obtained as a result of an unplanned consumption of hay by cow No. 4 in period 2. The first values obtained on this cow were in agreement with those shown by the other animals. Her next values revealed a marked improvement as regards the condition of acidosis. She had been observed on one or two occasions to be stealing hay from a neighboring cow not on the experiment. This hay was of the same composition as that fed during the third period and contained approximately 50 per cent of legumes, mostly clover. When a partition was put in to prevent hay consumption by cow No. 4, the succeeding values revealed the same state of acidosis as shown by the other cows.

In contrast to the above changes which occurred in the urine, it is noted in the last three columns of table 3 that the blood CO_2 combining capacity was not significantly altered by the feeding of phosphoric acid grass silage. Thus, it is apparent that the physiological changes reflected so clearly in the urine did not affect the alkaline reserve of the blood. This might lead to the conclusion that there was no essential disturbance of the acid-base balance were it not for the fact, as mentioned earlier, that three of the cows were in poor condition as a result of feeding the phosphoric acid grass silage alone. Their condition was markedly improved when urinary values were brought back to normal by the feeding of either hay or limestone.

DISCUSSION

While the alkaline reserve of the blood was not affected by feeding the acid grass silage as the sole ration, the effect on the condition of the cows, coupled with the marked changes in urine, suggests that grass silage ensiled with twenty pounds of phosphoric acid per ton is not a satisfactory sole ration for dairy cows but that some neutralizing agent should be included.

While it is generally assumed that urinary acidosis is of little physiological importance as long as the alkaline reserve of the blood plasma is maintained at a normal level, the present experiments suggest that a normal alkaline reserve is not in itself a sufficient criterion of a healthy condition. It is possible that more importance should be given to the urine picture than has usually been the case. Since cow No. 4 remained in good condition in spite of the acidosis revealed by the urine picture, it is also possible that some other factor is concerned for which no suitable chemical determinations have been made.

Some comment should be made on the fact that acid silage and grain proved satisfactory in Experiment I whereas it did not in Experiment III. In the first experiment, the silage contained a large amount of legumes and the grain mixture contained bone meal. In the third experiment, the silage was undoubtedly lower in lime and other basic constituents for the reason that it consisted almost entirely of grasses. Further, the grain mixture contained no bone meal. These differences in the rations fed might explain the differences in the results obtained. In the last experiment, the addition of grain following a period when the silage was the sole ration did not improve the condition of the animals. This is evidence that the condition was not due simply to a lack of a sufficient feed intake. On the other hand, since the addition of limestone alone was beneficial, it is apparent that the source of the trouble lay with the acidity of the silage.

SUMMARY

Three experiments involving a total of twelve animals are reported in which the changes in the acid-base relations in the blood and urine were studied with cows receiving phosphoric acid silage with and without hay or limestone. No changes in the blood or urine were observed, as compared with the data on a ration including corn and molasses silage, when the phosphoric acid silage was supplemented with either hay or limestone. The feeding of phosphoric acid grass silage alone resulted in marked urinary changes indicating acidosis but was without effect on the alkaline reserve of the blood. Three of the four animals developed an unthrifty condition when fed the silage alone. This condition was relieved and the urine values were brought back to normal by the addition of either hay or limestone. It is concluded that the phosphoric acid grass silage is a satisfactory feed for dairy cows when it is supplemented with hay containing an appreciable amount of legume or with limestone.

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BROOMCORN SILAGE FOR DAIRY CATTLE

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Illinois produced 29,000 acres, or approximately 13 per cent, of the 223,000 acres of broomcorn harvested in the United States in 1939. The acre yield is somewhat greater in Illinois than in the other five principal producing states which accounts for the fact that during the period 1928–1939 from 20 to 25 per cent of the harvested crop was grown in this state (1).

In harvesting broomcorn, the brush is removed by hand cutting shortly after it has emerged from the enveloping sheath at the top of the plant. At this stage the plant is still green, that is, it contains a large amount of moisture. As a rule, the brush is the only portion of the crop used and the remaining stalks, which may yield several thousand pounds of dry matter per acre, are plowed under. In some cases the crop, or a portion of it, is permitted to stand until maturity for the production of seed. It appears that the principal reasons the stalks are not used as forage as soon as the harvesting of the brush is completed are that the crop is unpalatable and supposedly poisonous to livestock.

Very few reports of experimental studies of the value or possibilities of broomcorn as a forage crop are to be found in the literature. Dowell and Friedemann (2) of Oklahoma conducted an investigation of the composition and digestibility of the immature broomcorn seed which is removed immediately after harvesting the brush. In this investigation they also studied the composition of the stover and the composition and digestibility of silage made from the green broomcorn plant. They report that broomcorn seed is less valuable as a feedstuff than the grain sorghum although the digestion coefficients of the two compare favorably. They report further that broomcorn silage kept in good condition, had a pleasant odor, was eaten with relish by sheep, and the digestibility of the dry matter of the silage was 51.5 per cent. They found that the amount of prussic acid in a sample of the whole plant harvested July 6th was 0.0098 per cent and the amount of tannin in the whole plant was about 0.2 per cent and in the seed 0.58 to 0.86 per cent.

The objects of the investigation reported herewith were: (a) to determine the yields of dry matter in the stalk portion of the broomcorn plant; (b) to find a suitable method for the preservation of the stalks as silage; and (c) to study the feeding value of the silage.

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EXPERIMENTAL PROCEDURE

Seed of the two varieties of broomcorn commonly grown in Illinois, namely, Black Jap and White Italian, was drilled in rows 3.5 feet apart in May, 1938. Adjoining the broomcorn plot, were plots of Orange sorgo and hybrid corn. The broomcorn was cultivated in the same manner as corn.

Beginning on August 9 and at intervals up to September 20, the crop from measured lengths of row was harvested and the yields of fresh matter and of dry matter were determined. The yields for August 9 and August 13 include the brush because on these dates many of the heads (brush) had not yet emerged and accurate separation was impossible.

Portions of the crop of each variety were ensiled in small containers at approximately two-week intervals, beginning on August 9. The containers were of galvinized iron construction and each held about 100 pounds of crop. The crop ensiled on August 9 included the brush but at later dates the brush was removed before passing the stalks through the silo-filling machine. The cut material was packed tightly by tramping.

The Black Jap variety reached about the right stage for brush harvest on August 22 while the White Italian variety appeared to need 7 to 10 days longer to reach this stage. A quantity of each variety was harvested on August 22, the brush removed, the stalks chopped by means of a silo filler and the cut material collected on a wagon. Small containers were filled as on previous dates. After weighing the remainder of the cut material, it was sprayed and thoroughly mixed with cane feeding molasses at the rate of 100 pounds of molasses to a ton of crop. Before application the molasses was diluted with an equal weight of water. A wood silo 4 feet in diameter and 10 feet deep was filled with the silage material of the Black Jap variety and another silo of the same size with White Italian. In both the small containers and wood silos the silage was covered with heavy asphalt roofing material and weighted with ground limestone.

The small containers were opened eight or nine months after filling. In most cases samples were taken for determination of dry matter and water-soluble acidity, but in some of the containers the silage was completely spoiled and no samples were taken. The wood silos were opened 15 to 16 months after filling and the silage fed to four Holstein cows during a double-reversal feeding trial. The experimental periods were four weeks in length with a one-week preliminary period and a one-week transition period. Comparison of broomcorn silage was made with corn silage.

EXPERIMENTAL RESULTS

It was found that both varieties of broomcorn gave large yields of fresh matter and of dry matter (table 1). The yields increased rapidly and continued to increase in the unharvested portion of the crop even after the usual stage for brush harvest. The yield of the Black Jap variety on Sep-

tember 20 was less than for the previous harvest date, a difference probably accounted for by the extensive breaking down of the plants brought about by the development of the heavy seed crop on the brush.

The White Italian is a larger-growing variety than the Black Jap, with larger and taller stalks. Twenty-five consecutive plants in a row of each variety were measured on September 20. The average length of the 25 harvested plants of the Black Jap variety was 125.6 inches and of the White Italian, 150.6 inches.

TABLE 1

Yields of fresh matter and of dry matter in broomcorn

Date of harvest	Variety	Dry matter in crop	Yield of crop per acre[1]	
			Fresh matter	Dry matter
		Percent	*lbs.*	*lbs.*
8– 9–'38	Black Jap	19.50	26390	5146
8– 9–'38	White Italian	16.70	28650	4875
8–13–'38	Black Jap	22.38	31125	6966
8–13–'38	White Italian	22.57	39000	8802
8–22–'38	Black Jap	28.51	23374	5589
8–22–'38	White Italian	27.92	31668	7368
8–27–'38	Black Jap	30.05	26200	7873
8–27–'38	White Italian	27.78	33500	9306
9–20–'38	Black Jap	32.31	20858	6739
9–20–'38	White Italian	34.36	28146	9671

[1] The yields reported for the harvests of August 9 and August 13 are for the entire harvested crop including the brush. The yields given for the other dates are for the harvested plants after removal of the brush.

The yields of the stalk portion of the broomcorn crop at the time the brush would normally be harvested, which in 1938 was the latter part of August, were nearly or fully as large as those of hybrid corn and Orange sorgo grown on adjoining plots. Several plots of hybrid corn harvested September 12 to 16, yielded from 7000 to 8000 pounds of dry matter per acre, while Orange sorgo harvested September 20 yielded 9070 pounds.

All of the lots of broomcorn ensiled without molasses were either completely spoiled or in very poor condition when the silos were opened (table 2). It appeared that insufficient acid was produced to bring about preservation. Also, broomcorn ensiled after the usual brush-harvest stage (August 22 to 31 in this investigation) seemed to lack sufficient amounts of moisture. While these results are only preliminary, they point strongly to the conclusion that for best results broomcorn stalks should be ensiled immediately following brush harvest and that liberal amounts of molasses or other preservative should be added.

The broomcorn ensiled in the wood silos on August 22 (Silos 2 and 3, Table 2) kept well, and had a good green color, strongly acid odor, and pleasant aroma. Several days were required for the cows to become accus-

TABLE 2

Results obtained in ensiling broomcorn at different stages of development

Silo No.	Date filled	Date emptied	Crop	Treatment	Dry matter in crop as ensiled	Dry matter in silage as emptied	Lactic acid in		Condition of silage
							Fresh silage	Dry matter of silage	
	1938	1939			%	%	%	%	
376	Aug. 9	May 2	Black Jap	None	19.50	14.11	.051	.36	Yellow; bad odor
424	Aug. 9	May 2	White Italian	None	16.70	10.17	.104	1.02	Very wet
444	Aug. 9	May 2	Broomcorn, 50% / Soybeans, 50%	None	18.10 / 18.92	13.27	.276	2.08	Too much moisture
449	Aug. 22	May 9	Black Jap	None	28.51	26.67	.025	.09	Too dry
628	Aug. 22	May 9	White Italian	None	27.92	22.95	.025	.11	Moldy
3	Aug. 22	Dec. 11	Black Jap	Molasses, 5%[1]	28.51	30.65	1.63	5.32	Good
2	Aug. 22	Nov. 13	White Italian	Molasses, 5%[1]	27.92	29.38	1.87	6.38	Good
17	Sept. 6	May 2	Black Jap	Molasses, 5%[1]	29.75	22.64	.....	.....	Dry; moldy
16	Sept. 6	May 2	White Italian	Molasses, 5%[1]	29.70	21.38	.079	.37	Dry; moldy
385	Sept. 6	May 2	White Italian, 50% / Soybeans, 50%	Water, 10%	29.70 / 28.29	18.93	.103	.54	Moldy
18	Sept. 6	May 2	White Italian, 50% / Soybeans, 50%	Molasses, 5%[1]	29.70 / 28.29	25.09	.896	3.57	Fair; acid odor
21	Sept. 20	May 9	Broomcorn	Molasses, 5%[1]	32.31	24.03	.....	.....	Very moldy
22	Sept. 20	May 9	Broomcorn	Molasses, 5%[1]	34.36	29.94	.....	.....	Very moldy

[1] Molasses was diluted with equal weight of water before application.

tomed to the silage and at no time during the experimental periods did they consume as much broomcorn silage as corn silage. Broomcorn stalks are hard and rigid and in the ensiling process the knives did not cut off all the stalks squarely but splintered many of them. These pieces were from two to five inches in length and remained hard and sharp even after 15 months

FIG. 1. Broomcorn Grown for Silage Investigation. Two rows of broomcorn photographed on August 20. At that time the heads (brush) of the Black Jap variety (left) were almost ready for brush harvest, while part of the heads (brush) of the White Italian variety (right) were just emerging from the enveloping sheath. Height of man, 6 feet.

in the silo. The refused portion of the silage, which amounted to 17.8 per cent of the silage fed, consisted mainly of these sharp pieces. It is likely that fine chopping by a machine with sharp knives would reduce the amount of silage refused.

The broomcorn silage seemed to have good nutritive value, as judged by its chemical composition (table 3) and by the milk production of 4 cows to which it was fed (table 4). The production of the cows declined more rapidly during the broomcorn silage periods than during the corn silage periods but this is accounted for in part at least by a lower feed intake when broomcorn silage was fed. So far as could be observed, the consumption of broomcorn silage had no harmful effects upon the cows.

The broomcorn silage was found to be high in fiber (table 3).

TABLE 3

Composition of broomcorn silage used in feeding trial

Variety of broomcorn	Dry matter	Ash	Total protein	Ether extract	Crude fiber	Nitrogen-free extract
	%	%	%	%	%	%
			Fresh basis			
Black Jap	30.65	2.50	1.41	0.44	10.74	15.56
White Italian	29.38	2.11	1.34	0.42	10.98	14.53
			Dry basis			
Black Jap	100.00	8.15	4.61	1.42	35.04	50.78
White Italian	100.00	7.18	4.55	1.41	37.36	49.50

TABLE 4

Summary of results of feeding broomcorn silage in comparison with corn silage to dairy cows

Kind of silage	No. of cows	Silage fed daily	Silage orts	Feed consumed daily per cow			Test of milk	Milk yield daily per cow[1]
				Silage	Hay	Grain		
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	%	*lbs.*
Broomcorn	4	21.1	3.8	17.3	22.0	13.9[2]	3.61	34.4
Corn	4	24.0	0.0	24.0	22.0	13.9[2]	3.72	35.4

[1] Milk energy in terms of 4% milk computed according to the formula .4 × milk (in pounds) + 15 × fat (in pounds).

[2] Includes 3 pounds of dried beet pulp.

SUMMARY

A small-scale investigation of the possibilities of using broomcorn for silage was conducted. Two varieties of broomcorn, Black Jap and White Italian, produced large yields of dry matter per acre, the amounts in the stalk portion of the plant at the usual brush-harvest stage being as large as the amounts in hybrid corn and in Orange sorgo harvested for silage. The White Italian is a larger growing variety than the Black Jap and gave larger yields of dry matter.

Broomcorn was ensiled at different stages of development. Best results were obtained in the case of broomcorn stalks ensiled at the usual stage for brush harvest.

Silage very low in acidity and with poor keeping qualities was found in all silos except those in which the cut crop was treated with molasses at the rate of 100 pounds per ton.

Broomcorn silage was found to be high in fiber content and hard splintered pieces several inches in length were refused by dairy cows. After becoming accustomed to the new feed, the cows ate the silage readily though

not as freely as when corn silage was fed, and produced only slightly smaller amounts of milk than during the corn silage periods.

The results obtained justify the conclusion that it is possible to make large amounts of silage having good keeping qualities and fair feeding value from the stalks of broomcorn, the portion of the plant now rarely utilized in any way except for plowing under.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Dr. O. R. Overman of the Dairy Chemistry Division in making the determinations of lactic acid reported in table 2. The analyses of broomcorn silage shown in table 3 were made by Messrs. J. M. Lindgren and John Herring of the Applied Chemistry Testing Laboratory of the University of Illinois.

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AGE, LIVE WEIGHT AND MILK-ENERGY YIELD IN ILLINOIS COWS

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In a previous article it was mentioned (1, footnote 6) that a plan of using live weight in connection with Dairy Herd Improvement Association records was under trail. This paper reports some of the results of that trial, dealing with all the Holstein (957) and Jersey (195) records available covering the first 8 monthly tests of the lactation without interruption. The records were all made under farm conditions, milking twice daily. It is impossible to deal with the records beyond the 8th month without in many cases encountering serious disturbances associated with advanced pregnancy and with management practice. Comparisons are simplified and biological meanings are clarified by dealing with the first 8 months of lactation (when the usual calving interval is 12 months) as compared with trying to deal with the whole lactation, or with fiscal year records. This fact was recognized by Gowen (2) as early as 1920, but the merits and advantages of the system are not yet generally appreciated.

ESTIMATE OF LIVE WEIGHT

Live weight of the individual cows was determined by use of a chest-girth live-weight tape sold by the New York Farm Bureau Federation, Ithaca, New York. The determination was made uniformly for each cow at the first visit of the tester after the cow calved, that is, within the first 30 days following parturition.

The tape used is marked at one centimeter intervals and each mark is labeled with a corresponding live weight in pounds. The following figures, taken from the tape, cover the range of size encountered in the present trial:

Chest girth, cm.	140	150	160	170	180	190	200	210	220
Live weight, lbs.	507	617	750	908	1085	1261	1437	1614	1790

AGE, LIVE WEIGHT AND YIELD

Only 255 of the Holstein records and 163 of the Jersey records include age of cow. The relation of age to live weight is shown graphically in figure 1; that of age to FCM,[2] in figure 2; that of live weight to FCM, in figure 3.

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[1] Acknowledgment is made to the several testers who secured the live weights and reported the records of the cows on which this paper is based.

[2] Symbols are used in this paper as follows:
FCM = milk-energy yield per day, pounds of 4% milk. (Calories of milk energy = 340 FCM.)

Table 1 proceeds to a more critical analysis of the separate and relative

TABLE 1

Influence of live weight (W) and age (A) on milk-energy yield (FCM) for cows under 7 years of age (Holstein, n = 199; Jersey, n = 140)

Breed	Partial lactation	Mean FCM	FCM = BW + DA		FCM = a + bW + dA			
			$\overline{W}B$	$\overline{A}D$	a	1000b	d	$\sigma_W b/\sigma_A d$
Holstein	1 mo.	37.7	30.7	7.0	−1.11	26.33	1.76	1.7
Jersey	1 mo.	36.1	35.6	0.5	6.92	32.56	0.70	3.8
Holstein	2 mos.	36.4	29.5	6.9	1.06	23.44	1.78	1.5
Jersey	2 mos.	35.3	35.5	−0.2	5.28	35.06	0.35	8.1
Holstein	3 mos.	35.0	28.5	6.4	3.83	20.15	1.71	1.3
Jersey	3 mos.	34.3	34.0	0.3	6.08	31.89	0.57	4.5
Holstein	4 mos.	33.7	28.2	5.4	4.51	19.29	1.47	1.5
Jersey	4 mos.	33.3	32.8	0.5	4.99	32.12	0.55	4.7
Holstein	5 mos.	32.6	27.7	4.8	5.43	18.10	1.34	1.5
Jersey	5 mos.	32.4	31.9	0.5	5.56	30.19	0.57	4.3
Holstein	6 mos.	31.5	27.2	4.2	7.23	16.09	1.22	1.5
Jersey	6 mos.	31.4	31.1	0.3	5.20	29.72	0.50	4.8
Holstein	7 mos.	30.4	26.8	3.5	8.54	14.53	1.09	1.5
Jersey	7 mos.	30.5	30.2	0.2	5.95	27.54	0.53	4.2
Holstein	8 mos.	29.2	26.1	2.9	9.89	12.84	0.96	1.5
Jersey	8 mos.	29.5	29.9	−0.5	6.48	26.37	0.37	5.8

$\overline{W}$ = mean W = 1211 pounds for Holstein cows; 822 pounds for Jersey cows.
$\overline{A}$ = mean A = 3.93 years for Holstein cows; 3.51 years for Jersey cows.
σ_W = 166.5 pounds for Holstein cows; 107.3 pounds for Jersey cows.
σ_A = 1.481 years for Holstein cows; 1.328 years for Jersey cows.

influence of age and live weight on FCM, for partial lactations of 1, 2, 3, 4, 5, 6, 7, and 8 months. In comparing the partial lactations of different lengths it should be borne in mind that differences may be affected by two factors: the effect of advance in lactation which may operate differently in different cows; the effect of summing or averaging, *e.g.*, the 1-month partial lactation is a single one-day test while the 8-month partial lactation is an average of 8 one-day tests spaced one month apart. Table 1 deals with cows less than 7 years of age in order to have substantially linear regression of FCM on age.

The last 4 columns of table 1 give the results of fitting the equation $FCM = a + bW + dA$ to the individual observations, that is, the observations are described by a plane which pivots at a point located at the mean of FCM, the mean of W, and the mean of A. The constant b in the equation describes

W = live weight of cow, pounds (determined within the first 30 days after calving)
A = age of cow at calving, years
n = number of cows or records
σ = standard deviation
$V = \text{variance} = \dfrac{\text{sum of squares of deviations from mean}}{\text{degrees of freedom}} = \sigma^2$

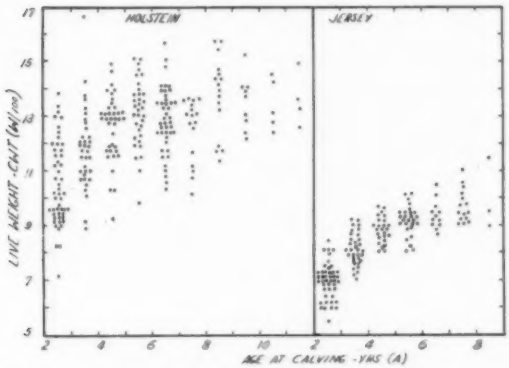

FIG. 1. Age and live weight. Omits 5 Holstein records above 11 years and 5 Jersey records above 8 years.

the slope which the plane takes under the influence of W independent of A; and the constant d describes the slope which the plane takes under the influence of A independent of W. The constant a is the mean FCM minus the sum of b times mean W and d times mean A.

A proper comparison of b and d as measures, respectively, of the influence of live weight and age on FCM yield requires weighting by the standard deviations (cf. 3, page 277). The last column of table 1 gives this weighted comparison and according to this point of view, it appears that weight is about 1.5 times as influential as age in the Holstein records, and about 5 times as influential in the Jersey records, in determining milk-energy yield.

Figure 6 gives a graphic representation of the planes, the equations of which are given in table 1, for the 1-month and 8-month partial lactations, and serves to give perhaps a more comprehensible picture of the relative influence of age and weight on milk-energy yield. For example in the Jersey 8-month record, as age increases by 5 years, there is an increase of 1.7 in FCM independent of weight; but as weight increases by 600 pounds, there is an increase of 15.8 in FCM independent of age. Clearly, live weight rather than age is the dominating influence on milk-energy yield.

Table 1 gives also the constants resulting from fitting the equation FCM = BW + DA. This equation, like the one above, describes the observations by a plane but in this case the plane pivots at the point of origin (instead of the point of means), that is, FCM = zero when weight and age are each zero. It assumes that FCM is a multiple of W plus a multiple of A. In case of the Jersey records, the multiple of W accounts almost entirely for FCM, leaving practically nothing left to be accounted for as a multiple of age. A similar condition prevails in the Holstein records, although slightly less extreme in the domination of live weight over age.

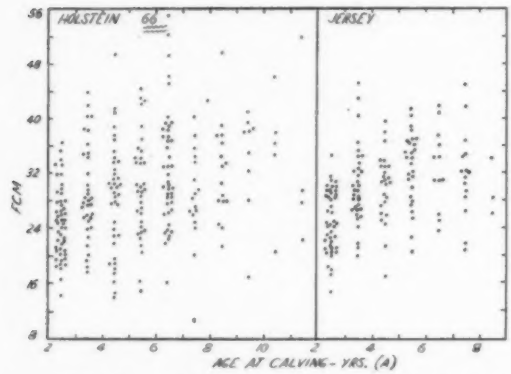

FIG. 2. Age and FCM for partial lactation of 8 months. Omits same ages as figure 1. Compare figure 4.

The above relations have very important significance in connection with the biological soundness of the much-used system of correcting milk records for age of cow. It is clear that correction for age is justifiable only in case the initial live weight is unknown, and then only insofar as weight is related to age and correctly portrayed by the particular set of age-correction factors that may be used. Age correction is an indirect allowance for initial live weight. It is biologically unsound and should be superseded by a system based on initial live weight, which can be readily used to eliminate the indirect influence of age as will be developed in the next section.

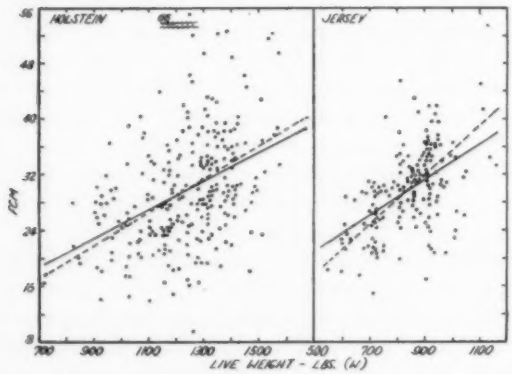

FIG. 3. Live weight and FCM for partial lactation of 8 months (ages known). The solid lines are fitted by least squares: Holstein, FCM = 4.6 + .0205W; Jersey, FCM = 8.1 + .0257W. The broken lines cut the origin and means: Holstein, FCM = .0242W; Jersey, FCM = .0353W. Power equation, FCM = bW^e, curve not shown: Holstein, e = .94; Jersey, e = .87. Compare figure 5.

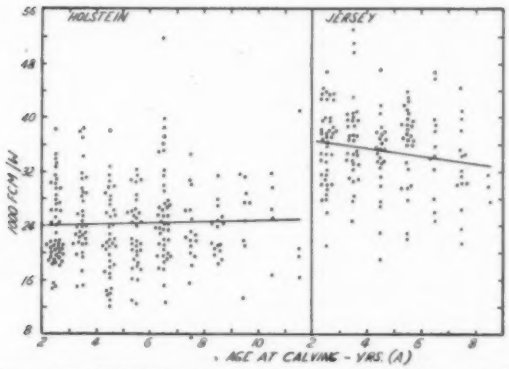

FIG. 4. Age and FCM/W for partial lactation of 8 months. Omits same ages as figure 1. Straight lines are fitted, including all ages, by least squares: Holstein, 1000 FCM/W = 23.7 + .12A; Jersey, 1000 FCM/W = 37.8 − .58A. Compare figure 2.

AGE AND FCM/W

The results (table 1) of fitting the equation, FCM = BW + DA, show that D is small and suggest that, so far as age is concerned, FCM = BW, practically. Hence, FCM/W = B, a constant so far as age is concerned, and a simple rational way to eliminate change in yield with age is to deal with FCM/W. Whether FCM/W is in fact independent of age may be tested directly by an analysis of variance (cf. 3, page 182). The test is to determine whether the variance of FCM/W is significantly greater between age groups than it is within age groups. If it is significantly greater, age differences must be considered; if it is not significantly greater, age differences may be disregarded. The results are given in table 2.

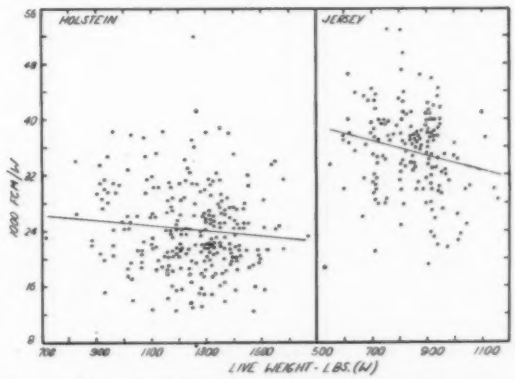

FIG. 5. Live weight and FCM/W for partial lactations of 8 months (ages known). Straight lines are fitted by least squares: Holstein, 1000 FCM/W = 29.0 − .0038W; Jersey, 1000 FCM/W = 44.0 − .0101W. Compare figure 3.

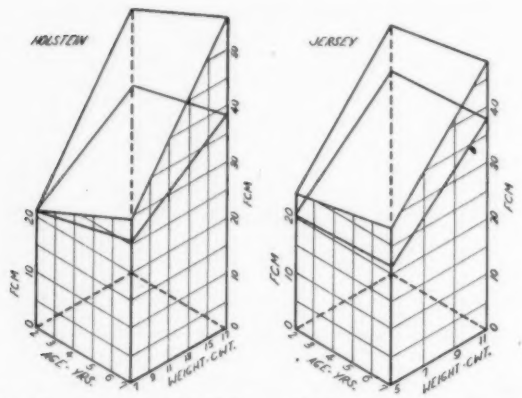

FIG. 6. Age, live weight and FCM for partial lactations of 1 month (upper plane) and 8 months (lower plane). Constants of the equations of the planes, FCM = a + bW + dA, are given in table 1.

TABLE 2

Analysis of variance, records classified by age
(1 year intervals, age classes, 2–13 years)

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 255								
V, between groups	121.7	124.7	100.3	84.4	73.3	59.5	55.3	44.6
V, within groups	79.7	73.1	66.6	58.5	50.9	46.0	42.6	37.6
F[a]	1.53	1.71	1.51	1.44	1.44	1.29	1.30	1.19
Regression[b]	+0.40	+0.39	+0.36	+0.35	+0.28	+0.23	+0.18	+0.12
Jersey, n = 163								
V, between groups	111.3	89.2	87.1	84.8	79.9	69.4	60.0	53.0
V, within groups	74.1	53.8	45.4	44.2	41.3	38.5	37.3	36.5
F[a]	1.50	1.66	1.92*	1.92*	1.93*	1.80	1.61	1.45
Regression[b]	−0.55	−0.50	−0.53	−0.52	−0.53	−0.54	−0.54	−0.58

[a] Test of significance, * Significant at the 5 per cent level.
[b] Change in 1000 FCM/W for an increase of 1 year in age.

Taking the Holstein 8-month records in table 2 as an example, the ratio of the larger variance to the smaller, designated as F, is 1.19. The degrees of freedom are respectively 11 and 243. Reference to Snedecor's (3, page 184) table shows that in this case an F value of 1.83 is required at the 5 per cent level of significance. The F value, 1.19, is far below the 5 per cent level of significance and accordingly we may conclude there are no significant differences between age groups with respect to FCM/W. A similar conclusion applies to the Jersey 8-month records.

The regression of FCM/W on age, table 2, is positive in the Holstein records and negative in the Jersey records. This may be due to a breed

difference, but a more likely interpretation seems to be that the true regression in both breeds is zero. FCM/W may be used freely without regard to age of cow, and affords a simple rational solution of the "age-correction problem" which may be presumed to apply to dairy cows in general.

TWO RECORDS OF THE SAME COW

Among the records with age given only 12 Holstein cows and 1 Jersey have two records each. These records for the Holstein cows are shown in table 3. Age-corrected (A–C) FCM is based on the factors used by the Bureau of Dairy Industry in the proved sire work.

There are several points of interest in table 3. The variance between cows is much greater than that within cows for each of the attributes of lactation considered, *viz.* FCM, age-corrected FCM and FCM/W. With respect to FCM and age-corrected FCM cow No. 12 plays a prominent part as shown by the variance values (between cows) including and excluding this individual. With respect to FCM/W the variance values (between cows) are not very much affected by the presence or absence of cow No. 12.

TABLE 3

Correlation between two records of the same cow with respect to FCM, age-corrected (A–C) FCM and FCM/W for partial lactation of 8 months

Cow No.	Age		W		FCM		A–C FCM		1000 FCM/W	
	1	2	1	2	1	2	1	2	1	2
1	2	3	945	1224	31.9	42.0	38.6	46.3	33.8	34.3
2	2	3	1020	1166	33.6	36.4	40.7	40.1	32.9	31.2
3	3	4	1110	1074	24.2	24.1	26.7	25.0	21.8	22.4
4	3	4	1173	1496	21.3	31.7	23.5	32.8	18.2	21.2
5	4	5	926	979	27.9	30.2	28.9	30.4	30.1	30.8
6	4	5	1194	1415	30.6	29.0	31.7	29.2	25.6	20.5
7	4	5	1296	1208	31.4	26.5	32.5	26.7	24.2	21.9
8	4	5	1384	1243	28.9	23.3	29.9	23.5	20.9	18.7
9	5	6	1261	1402	35.4	36.1	35.6	36.1	28.1	25.7
10	6	7	1085	1120	37.6	33.8	37.6	34.0	31.7	30.2
11	6	7	1208	1279	28.8	26.9	28.8	27.1	23.8	21.0
12	12	13	1550	1544	52.3	51.8	57.8	58.5	33.7	33.5
Cows 1–11	V, between cows				40.83		64.49		54.00	
	V, within cows				13.63		11.46		2.95	
	F[a]				3.00*		5.62**		18.28**	
	Intraclass correlation				0.50*		0.70**		0.90**	
Cows 1–12	V, between cows				114.32		172.32		59.07	
	V, within cows				12.50		10.53		2.71	
	F[a]				9.14**		16.36**		21.81**	
	Intraclass correlation				0.80**		0.88**		0.91**	

[a] Test of significance; * Significant at the 5 per cent level; ** Significant at the 1 per cent level.

The intraclass correlation in table 3 is a measure of consistency in the magnitude of the two records with respect to the given attribute, assuming diversity exists between the individual cows. The records are most consis-

tently alike for the same cow and unlike for different cows in the case of FCM/W. This in itself need not necessarily mean that FCM/W is the best measure of lactation. The question turns too much on the true degree of likeness actually existing among the several cows involved. To measure likeness some quantitative criterion must be used and this necessitates the use of a philosophy of some sort.

The problem may be illustrated by considering the data for cow No. 12 in table 3. By the age-correction philosophy this cow is by far the best one of the lot. By the FCM/W philosophy she is distinguished from the others in being a large cow (1550 pounds) but not in any extraordinary dairy tendency or proclivity to lactation, being exceeded slightly by one and nearly equalled by 3 others in FCM/W. Cannot the biological aspects of milking capacity in dairy cattle be put on a more fundamental basis by dealing with size of cow and yield per unit size, rather than with yield alone or age-corrected yield? This raises the important practical and biological questions: Is FCM/W independent of W in these records? If not, can we expect to develop dairy cows to the point where FCM/W is independent of W, that is, to the point where milk-energy yield, or lactation work, is proportional to initial live weight?

LIVE WEIGHT AND FCM/W

Dealing first with the records where age is known, table 4 presents an analysis of variance to test the significance of differences between live weight groups with respect to FCM/W in a manner similar to that used in table 2 for age.

In the Holstein records, by the F test the differences between live weight groups with respect to FCM/W are below the 5 per cent level of significance for each of the 8 periods of partial lactation, 1 to 8 months. For the 1-month,

TABLE 4

Analysis of variance, records (age known) classified by live weight (100-pound intervals, weight classes 500–1600 pounds)

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 255								
V, between groups	48.9	29.1	35.2	38.5	37.3	37.8	39.9	45.6
V, within groups	82.7	77.0	69.3	60.4	52.4	46.9	43.3	37.6
F[a]	1.69	2.65	1.97	1.57	1.40	1.24	1.09	1.21
Regression[b]	+0.24	+0.17	+0.03	−0.02	−0.10	−0.18	−0.27	−0.38
Jersey, n = 163								
V, between groups	125.9	54.9	79.5	83.6	93.1	93.8	93.9	94.3
V, within groups	74.5	56.0	46.8	45.3	41.7	38.3	36.5	35.3
F[a]	1.69	1.02	1.70	1.85	2.23*	2.45*	2.57*	2.67*
Regression[b]	−0.56	−0.48	−0.71	−0.65	−0.74	−0.78	−0.84	−1.01

[a] Test of significance, * Significant at the 5 per cent level.
[b] Change in 1000 FCM/W for an increase of 100 in W.

2-month, and 3-month partial lactations the regression is positive, that is, the large cows yield more milk energy per unit initial live weight than the small cows. The regression changes progressively as the length of partial lactation increases.

In the case of the Jersey records, the regressions are negative throughout and show a progressive change similar to that for the Holsteins. For partial lactations of 1 to 4 months, the difference between live weight groups is below the 5 per cent level of significance; for partial lactations of 5 to 8 months, the difference is above the 5 per cent level but below the 1 per cent level of significance.

The evidence of table 4 is that FCM/W is substantially independent of W for short periods of lactation but a negative correlation tends to appear as the length of period used increases.

Dealing with all the records, table 5 presents an analysis of covariance designed to show the relation between W and FCM/W, in total, and segregated as between herds and within herds. In the Holstein records the total correlation starts as + .03 in the 1-month records and finishes as – .12 in the 8-month records. While the latter is significant at the 1 per cent level, the actual decrease in 1000 FCM/W is not large, amounting to only .4 for an increase of 100 in W. In the Jersey records the total correlation between W and FCM/W is below the 5 per cent level of significance throughout. These total correlations suggest the conclusion that FCM/W is independent of W.

When the correlation between W and FCM/W is considered as between herds and within herds, there is throughout a relatively large positive correlation as between herds and a relatively small negative correlation within herds. That is, large-cow herds yield more milk energy per unit size of cow than do small-cow herds. But within a herd, there is a tendency for a large cow to yield less milk energy per unit size than her small herd mates.[3]

[3] The negative regression of FCM/W on W within herds is of an order of magnitude that might be expected on the theory that milk-energy yield is proportional to the 3/4 power of W. This suggests the interpretation that real size, "metabolic body size" or "physiologic weight," is proportional to the 3/4 power of W rather than to W itself. The positive and substantial regression of FCM/W on W as between herds would be explained as due to differences in herd management with a pronounced tendency for herds made up of large cows to have more favorable conditions than herds made up of small cows. An alternative to the 3/4 power interpretation is the interpretation (here preferred) that the milking potentialities of the large cows within a herd are not as fully realized as in the case of the smaller herd mates.

The fact that the relation between W and FCM/W is different between herds than it is within herds raises a question in connection with figure 6. Is the relative influence of weight and age on milk-energy yield the same within herds and between herds as it is in total as pictured in figure 6? The present records are not adequate to deal with this question.

TABLE 5

Analysis of variance and covariance with respect to W and FCM/W, records classified by herds (30 Holstein herds, mean W, 986 to 1395; 21 Jersey herds, mean W, 745 to 955)

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 957								
V, between herds	362	348	322	295	274	251	230	217
V, within herds	56	47	41	37	34	30	28	26
F[a]	6.46**	7.40**	7.85**	7.97**	8.06**	8.37**	8.21**	8.35**
Correlation[b]								
Total	+0.03	−0.01	−0.02	−0.04	−0.05	−0.08*	−0.10**	−0.12**
Between herds	+0.33	+0.28	+0.23	+0.23	+0.23	+0.24	+0.23	+0.23
Within herds	−0.03	−0.07*	−0.08*	−0.10**	−0.12**	−0.15**	−0.17**	−0.20**
Regression[c]								
Total	+0.13	−0.03	−0.09	−0.15	−0.21	−0.28	−0.34	−0.40
Between herds	+1.60	+1.31	+1.06	+1.02	+0.96	+0.98	+0.89	+0.86
Within herds	−0.17	−0.30	−0.31	−0.38	−0.44	−0.53	−0.59	−0.65
Jersey, n = 195								
V, between herds	210	187	164	164	157	138	129	121
V, within herds	64	47	39	36	33	31	29	28
F[a]	3.28**	3.98**	4.21**	4.56**	4.76**	4.45**	4.45**	4.32**
Correlation[b]								
Total	−0.02	−0.01	−0.03	−0.02	−0.03	−0.04	−0.06	−0.09
Between herds	+0.51*	+0.50*	+0.48*	+0.47*	+0.45*	+0.45*	+0.45*	+0.43
Within herds	−0.18*	−0.18*	−0.20*	−0.19*	−0.21**	−0.22**	−0.24**	−0.28**
Regression[c]								
Total	−0.13	−0.04	−0.16	−0.10	−0.18	−0.22	−0.29	−0.45
Between herds	+4.32	+4.00	+3.57	+3.47	+3.25	+3.08	+2.96	+2.73
Within herds	−1.25	−1.06	−1.10	−1.00	−1.05	−1.05	−1.11	−1.26

[a] Test of significance, * Significant at the 5 per cent level, ** Significant at the 1 per cent level.
[b] Correlation between W and FCM/W.
[c] Change in 1000 FCM/W for an increase of 100 in W.

FATNESS AT CALVING AND FCM/W

At the time of determining live weight of the individual cows in the present trial the tester also made a score of the cow intended to describe her condition of flesh. The scheme of scoring was based on a scale from 1 to 9, in which an excessively thin condition was scored as 1 and an excessively fat condition was scored as 9, all on the somewhat indefinite verbal expressions: thin minus = 1, thin = 2, thin plus = 3, medium minus = 4, medium = 5, medium plus = 6, fat minus = 7, fat = 8, fat plus = 9. Scores were made as above for 761 of the 957 records of Holstein cows, and 183 of the 195 records of Jersey cows. The results are given in table 6. The differences between

TABLE 6

Analysis of variance, records classified by score of cow with respect to fatness at calving (see text)

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 761								
V, between groups	89.8	72.3	66.2	51.6	49.3	34.4	35.2	37.5
V, within groups	63.8	55.0	49.0	44.0	41.5	36.7	33.9	31.8
F[a]	1.41	1.31	1.35	1.17	1.19	1.07	1.04	1.18
Regression[b]	+0.66	+0.51	+0.46	+0.36	+0.31	+0.15	+0.12	+0.07
Jersey, n = 183								
V, between groups	78.1	31.1	25.0	27.0	25.9	27.2	27.5	36.1
V, within groups	79.4	61.4	52.2	49.1	45.5	41.5	39.2	37.1
F[a]	1.02	1.97	2.09	1.82	1.76	1.53	1.43	1.02
Regression[b]	+1.03	+0.55	+0.98	−0.12	−0.36	−0.57	−0.64	−0.83

[a] Test of significance, all are below the 5 per cent level.
[b] Change in 1000 FCM/W for an increase of 1 in fatness score.

groups on the basis of fatness at calving are well below the 5 per cent level of significance in all cases. Hence it appears that FCM/W is independent of fatness of the cow at calving.

DISCUSSION

The results of the present investigation bear directly on the postulates previously advanced (4) that FCM/W for the 8-month partial lactation is independent of:

1. Service period and length of lactation.
2. Age of cow at start of lactation.
3. Fatness of cow at start of lactation.
4. Live weight of cow at start of lactation.
5. Composition of milk for the lactation.
6. Breed of cow.

Of these postulates 1 is automatic and 5 has been heretofore amply demonstrated for various other records. Postulates 2 and 3 are good on the evidence of the present paper. Postulate 4 is good within either the Holstein or Jersey breed on the present evidence. Postulate 6 is still a question.

In the present records the Jersey breed is far above the Holstein breed on the basis of FCM/W. This is interpreted to mean that the present Jersey cows are superior to the Holstein cows, either in point of inherent proclivity to lactation (FCM/W), or in point of management, or both (probably both). There can hardly be any question as to the superiority of the Jersey records since the 822-pound average Jersey of table 1 produced in the 8-month partial lactation an absolute 10,030 Calories of milk energy per day (estimated as 29.5×340) while the 1211-pound average Holstein of table 1 produced an absolute 9,928 Calories per day (estimated as 29.2×340). That is, the small Jersey did more work in lactation than did the large Holstein.

It is clear that the performance of the Jersey cows excels that of the Holstein cows in the present data; whether it excels to the extent indicated by FCM/W may be open to question. The dairy cow is undoubtedly undergoing evolution under the pressure of artificial demands and selection. Can that evolution be pressed to the point of equality with respect to FCM/W as between large and small breeds of cows? Apparently, it has already reached that point within the large breed, or within the small breed, (cf. 1, footnote 11).

As between the use of FCM/W or the use of age-corrected FCM the case is entirely clear. Age is often unknown, while live weight is always determinable in a system of record keeping. Furthermore, the physiological basis of increase in yield with age appears to be associated with increase in size of the cow with age, age of itself being a practically negligible factor. The use of age correction is so widespread and deeply rooted that any question of its soundness may meet with opposition, and a demand for more extensive investigation before acceptance. It should be held in mind that the argument here presented is based on initial live weight[4] (within the first 30 days after calving, preferably about the 3rd–5th day) and on milk-energy yield for the first 8 months of the lactation.

SUMMARY AND CONCLUSIONS

The data used consist of 957 records of Holstein cows and 195 records of Jersey cows for partial lactations of 1, 2, 3, 4, 5, 6, 7, and 8 months in Illinois Dairy Herd Improvement Associations. The records are computed to a daily milk-energy basis in terms of 4 per cent milk in pounds (FCM). Live weight in pounds (W) within the first 30 days after calving is a part of the record. Age is known for 255 Holstein records and 163 Jersey records.

[4] Where live weight is estimated after the close of the record period, as for example, in the extensive early 12-months Register of Merit records of the Jersey breed, an entirely different relation appears between age, weight and yield, as compared with the relation found in the present work. Final live weight and initial live weight may be quite different and have different biological significance. It is here considered essential to deal with initial live weight.

The data are studied by graphic portrayal, by fitting equations and by analysis of variance.

By fitting the equation, $FCM = a + bW + dA$, where A = age, it is shown that the influence of age on FCM independent of weight is entirely negligible in the Jersey records and practically so in the Holstein records (see figure 6). By fitting the equation, $FCM = BW + DA$ it is shown that D is practically zero in the Jersey records and very small in the Holstein records. Hence, $FCM = BW$ or $FCM/W = B$, a constant so far as age is concerned, and FCM/W should be independent of age. Analysis of variance applied to the records classified by age shows that differences between age groups (2 to 13 years, by years) are in fact well below the 5 per cent level of significance.

It is concluded that the use of age correction factors is justified only in case W is unknown and then only insofar as W is associated with age and correctly portrayed by the factors used. FCM/W may be used freely without regard to age of cow. As rapidly as feasible initial live weight at each lactation should be made a part of all dairy records and FCM/W (or similar principle) should supersede the biologically unsound principle of age correction.

Analysis of variance and covariance applied to the records classified by W shows that differences between the live weight groups with respect to FCM/W are below the 5 per cent level of significance for the shorter partial lactations but become increasingly significant up to the 8-month partial lactation. In general, the regression of FCM/W on W is negative and in the gross amounts to a decrease of about .4 in 1000 FCM/W for an increase of 100 in W. However, as between herds an opposite tendency prevails and as between Jersey herds, there is an increase of 2.73 in 1000 FCM/W for an increase of 100 in W.

It is concluded that as between cows of different live weights in either the Holstein or Jersey breed, FCM/W affords an equitable criterion of dairy merit or development.

Analysis of variance applied to records classified by fatness of cow at calving shows no significant differences between groups, and it is concluded FCM/W is independent of fatness of cow at calving.

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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

479. Official Body and Texture Criticisms of Dairy Products Judged in the National Contest. G. M. Trout, William White, P. A. Downs, M. J. Mack, and E. L. Fouts, American Dairy Science Association Committee on the Judging of Dairy Products.

A study of the official body and texture criticisms of butter, cheese and ice cream samples used in the Students' National Contest in the Judging of Dairy Products during the fourteen year period from 1926 to 1939 inclusive shows that body defects of butter were encountered but a few times, whereas in cheese and ice cream they were encountered in the greater majority of cases. Predominating body and texture criticisms were: for butter, "leaky"; for cheese, "open," "weak," "pasty" and "mealy"; and for ice cream "coarse" and "weak" for the frozen product, and "curdy" for the melting appearance. Approximately two body and texture criticisms were used for each sample of cheese and ice cream so criticized whereas one was sufficient in the case of butter.

480. Preliminary Observations on Chemical Changes of Rumen Ingesta with and without Urea. M. I. Wegner, A. N. Booth, G. Bohstedt, and E. B. Hart. From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Dry matter, fiber, ether extract, ammonia nitrogen, non-protein nitrogen, and total nitrogen have been determined on rumen contents of a fistula heifer on a basal ration of corn silage, timothy hay, and corn and oats with and without added urea. The level of total nitrogen and fiber found in the rumen material is distinctly higher than in the ration fed. Urea nitrogen or ammonia nitrogen when ingested as 1 to 5 per cent of the dry matter of the ration fed disappeared from the rumen in 4 to 6 hours after feeding. A definite increase in the percentage of protein nitrogen of the rumen ingesta was produced by adding 5 per cent urea to a low nitrogen basal ration.

481. The Relation of Mastitis to the Level of Ascorbic Acid and Certain Other Constituents in Milk. E. P. Reineke, E. R. Garrison, and C. W. Turner, Missouri Agr. Exp. Sta., Columbia.

The ascorbic acid content of the milk from ninety individual quarter samples from nineteen cows in the University of Missouri dairy herd was correlated with the incidence and severity of mastitis and the level of chlorides in the milk.

In early stages of mastitis the ascorbic acid of the milk was reduced about 10 per cent and in advanced cases it was reduced by as much as 30 to 50 per cent. It was shown that there is a trend toward an inverse relationship between ascorbic acid and chloride in mastitis milk and also in the milk produced following perfusion of the mammary duct system with a hypertonic solution.

Long chain streptococci isolated from mastitis milk were shown to actually retard the rate of oxidation of ascorbic acid *in vitro*. A higher ascorbic acid oxidase content in mastitis milk was also ruled out as a factor causing the reduction in ascorbic acid.

The theory is advanced that the effect of mastitis upon the level of ascorbic acid in milk is exerted indirectly by producing a change in the selective permeability of the milk secreting cells in relation to the osmotic equilibrium existing between blood and milk.

482. Antioxygenic Fractions of Oat and Soya Bean Flour. C. D. DAHLE AND D. H. NELSON, Dairy Department, The Pennsylvania State College.

An attempt was made to determine the active fraction of two cereal antioxidants—namely, oat and soya bean flour, when used in pure butterfat. Aqueous, acetone, alcohol, ether, and hexane extracts were made. A phospholipid extract was obtained from the flours and studied in this connection.

In the trials studied the phospholipid and alcohol extracts exhibited the greatest anti-oxygenic properties in pure butterfat held at 60° C.

483. The Riboflavin Content of Milk as Influenced by Diet. PAUL JOHNSON, L. A. MAYNARD, AND J. K. LOOSLI, Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.

Experiments with cows, involving 460 determinations made over a period of 9½ months, showed clearly that the riboflavin content of milk can be influenced only to a limited extent by the diet. When cows were transferred from pasture to a ration of natural feeds selected to be very low in riboflavin its content in the milk decreased about 25 per cent. Increasing the riboflavin intake 30 to 50 per cent by feeding a molasses-yeast by-product caused only a temporary increase in the concentration of riboflavin in the milk. A winter ration consisting of good quality hay, acid grass silage and a grain mixture maintained the milk riboflavin at the pasture level.

Goats fed a riboflavin-free purified diet continued to secrete large amounts of riboflavin in the milk, indicating this factor is not a dietary essential for lactation in the goat. No consistent difference was observed in the milk yield or the riboflavin concentration when the purified diet was supplemented with a molasses-yeast by-product supplying riboflavin. Like-

wise, no advantage in milk secreted was noted between a thiamin-deficient purified diet and one adequate in it and other factors of the B-complex.

The data from both cows and goats indicate that there is an inverse relation between the milk yield and the riboflavin concentration of the milk.

BOOK REVIEWS

484. The Butter Industry. O. F. HUNZIKER. Third Edition, 1940, 780 pages, illustrated. Published by the Author, La Grange, Ill. Price $6.50.

Since the publishing of the second edition of this authoritative work in 1927, developments along many lines have taken place in the creamery industry, and Dr. Hunziker brings this comprehensive and exhaustive treatise up-to-date with his usual unequaled thoroughness. New material has increased the volume to 769 pages of text, more than one-fourth larger than the second edition. The organization of the book follows the same general plan.

A new chapter with much new material on steam power, refrigeration and water-tempering systems, is added. A consideration of corrosiveness of refrigerating brines makes this phase of the work more complete, along with a discussion of boilers and feed waters.

An entire chapter is devoted to creamery equipment—materials of construction, mechanical care and use. The sanitary care of the churn, one of the most important phases of sanitation in the production of butter of good bacteriological keeping quality, is given thorough treatment, and the factory man will find such material extremely helpful. The hope of the sanitarian—the all-metal churn—receives attention along with the no-roll wood churns of recent date.

In the discussion of the classification of cream grades the author takes his accustomed strong stand on the necessity for cooperation among all components of the industry in paying for cream on a quality basis in order for progress in cream improvement and quality of butter to occur. A system of grading cream looking toward the establishment of a national brand of American butter is suggested.

The introduction in recent years of steam-injection pasteurization has added new material to the chapter on pasteurization, and the increased importance of fat losses in buttermilk with this system of pasteurization is discussed. Recent developments in the treatment of cream for the removal of objectionable flavors and odors are described and the author draws on his wide experience in this field in evaluating the merit of the various procedures.

The chapter on starter-making and cream ripening is valuable in bringing the reader up-to-date on the essentials of the progress that has been

made in recent years with the recognition of the role of diacetyl and acetylmethylcarbinol in the production of the desired flavor in butter.

The churning process itself, the focal point of butter-making, is dealt with exhaustively from theoretical as well as practical viewpoints. The pH of butter and its relation to churning acidity of the cream are discussed, as well as the significance of the pH of butter in the determination of keeping quality in cold storage.

The chapter on packaging butter brings this important subject up-to-date. Apparently, foreign countries have gone further with the packaging of butter direct from the churn than the U. S. Undoubtedly, this phase of the butter industry will see further developments in the next few years. The chapter on "Markets and Marketing of Butter" contains new material on the intricacies of trading in "futures," and the new "Official U. S. Standards for Quality of Creamery Butter," effective April 1, 1939, are given in detail.

"Butter Defects" occupies, as a chapter, the prominent position in point of increased size and detail of development that its importance warrants. The student and research worker will find in this chapter a thorough development of the problems of butter quality that face the industry, and in the presentation of unsolved problems, a stimulus to continued and thorough investigation. "Surface taint" and allied defects in flavor of butter, due to bacterial decomposition of the protein, have been subjected to much careful investigational work in recent years. The rise of this type of defect in butter in this country, and the present status of the problem are presented with definite suggestions as to means of prevention. The research worker in bacteriology will find this subject intriguing. New information on defects in body and texture is made available, especially for the prevention of crumbly and sticky body, so commonly found in winter butter in this country.

In format the third edition is patterned after the second and presents a physical appearance that will be a credit to any library. The broad scope of the work, embracing as it does all phases of the butter industry, assures that it will follow its predecessor in an international circulation among practical buttermakers, students, research workers, and officials of law-enforcing agencies.

W. A. CORDES

485. Applied Mycology and Bacteriology. L. D. GALLOWAY AND R. BURGESS. Price $4.00, 186 pages, illustrated. Distributed by Chemical Publishing Co., Inc., 148 Lafayette St., New York, N. Y.

This book presents a brief summary of the recognition, methods of handling, and control of microorganisms of special interest to industry. Due to the breadth of the field treated the discussions necessarily omit detailed information on many of the subjects included. Numerous references,

however, enable the reader to use this book as an introduction to the various fields of applied mycology and bacteriology.

The contents of the book are as follows: Part I. Fungi, bacteria, apparatus and sterilization, isolation and examination of microorganisms, culture media and stains, metabolism of microorganisms, control of microorganisms. Part II. Food industries, fermentation industries, textile industries, hygiene, agricultural applications, miscellaneous.

The practical dairyman and research worker in the dairy field will find only a brief treatment of their subject. Chapters on food industries, fermentation industries and particularly textile industries present very good general discussions.

This book is well written throughout. It should prove useful to the student as a supplementary text and to the industry as a guide to information on the recognition, methods of handling, and control of microorganisms in the food industry. P.R.E.

486. Milk Distribution as a Public Utility. W. P. MORTENSON, Univ. of Wis. July, 1940. 213 pages. Price $2.50. Published by University of Chicago Press, Chicago, Ill.

The reviewer is desirous of stating at the outset that in the light of its title, this book definitely is not of the Union Square soap-box oratory type. The author has based his discussion of the subject with facts in the study of operating costs of many milk plants, mainly in Wisconsin. Nor is the book a ponderous statistical handbook. It contains a number of selected summarized tables that present the points the author is making.

As a background there is an interesting review of the economic philosophies that have lead to the type of dairy organizations, both large and small, such as we know today. Whether these businesses fall into the category of public utilities is defined by describing what constitutes a public utility. Operating a milk business in a community as a utility means, concisely, consolidating the motions in the business. The author has discussed specifically, the potential savings that would evidently be possible by unifying the operations of the several plants in a municipality. The estimates are derived from the knowledge of what men, machinery and time are doing, and what these factors reasonably can do. The potential efficiencies are cited for the various items of cost, as plant equipment, plant labor, distribution and so forth. Nor are the probabilities of not making savings overlooked in this review. An interesting confirmation of the appraisal of the benefits of consolidation is in part shown by the review in a chapter of the profits of large and small companies for various years.

What are the possibilities of legal sanction of unification of the milk business? The author has presented in a chapter excerpts of cases (principally U. S. Supreme Court) involving other businesses of the same general

nature determining whether they fell into groups having great liability to public interest. The Supreme Court has not ruled on the legality of the exclusive-franchise for operation of a unified milk business. There are indicated the legal precedences which might make such regulatory action possible, and the factors which favor or dis-favor this possibility. The potential problems of either public ownership, and public utility operation of the milk business are cited. The inherent differences that exist between the normal utilities, as gas, electric and water companies and a unified milk business, and the relative effect of factors on the operation of these businesses are presented. Lastly, who stands to benefit by the institution of a unified milk distribution system? Certain groups, as for example organized producers, it is stated, are better off with the competitive rather than with the unified system.

Technically, there is little doubt that unification of operations could bring about reduction of costs from $1\frac{1}{2}$ to $2\frac{1}{4}$ cents per quart. But the social implications, the selection of capable and honest management, freedom from political interference, price determination and the like are activities which are unpredictable, and which may defeat any technical advantages of unification. The outlook for a unified system is concluded. This book is readable, understandable, impartial in presentation. It is of value to all associated with the dairy industry, and those in or aspiring to public office, as an excellent review and discussion of the contemporary social problem of the industry. K.G.W.

487. Fruit Pectins. Their Chemical Behavior and Jellying Properties. C. L. HINTON, Dept. of Scientific and Industrial Research, Great Britain. Food Investigation Special Report No. 48. 96 pages, Price $1.75. Published by Chemical Publishing Co., Inc., 148 Lafayette St., New York.

The information presented is based on work conducted by the staff of the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades. The divisions in the book include the constitution, characterization, measurement of jelly-forming capacity and chemical properties of pectins. The data on pectins from five fruits (oranges, lemons, apples, gooseberries and strawberries) is presented. The effects of heating, acids, alkalies, and salts, the methods of extraction and the action of pectase on the various pectins is reviewed. The data is excellently illustrated by the use of graphs (13) and tables (42). The jellying power of pectins is dependent upon its inherent quality (some are naturally weak, others naturally strong), the degree of heating to which it has been subjected, and effects of treatment with strong acids at ordinary temperatures or less. The jellying power of pectins as exhibited by the pectins in jellies is further affected by the degree of de-esterification caused by acids or pectase action,

pH conditions as fixed by buffering constituents or neutralization of the free acid of the pectins, and the beneficial effect of some salts (calcium chloride, for example, increases the pH range over which a pectin makes suitable jellies).

There is no specific reference to the use of pectins in milk products. Nevertheless, the properties of the pectins are very well covered and the information will be of use to manufacturers of, and research workers in, products such as fruit ice cream pies, fruit ice cream flavors, and special products as spreads. K.G.W.

BACTERIOLOGY

488. Oxidase-Positive Bacteria in Dairy Products. C. H. CASTELL. Can. Dairy and Ice Cream J., *19:* 5, 26. 1940.

"Oxidase-producing" bacteria are responsible for the spoilage of fatty foods by oxidation. They also hydrolyze the fat and bring about rapid decomposition of proteins. Those giving very strong reactions included all the members of the Pseudomonas and Achromobacter genera. Individual members showed the following defects in cream and butter: potato odor, surface taint, cheesiness, limburger, putridness and rancidity. Organisms giving a less marked reaction were those of the Brucellus and Alcaligenese genera and the Gram negative organisms from soil and diseased plants. Oxidase-negative organisms included most coccus types and a large majority of spore-forming bacteria. Most molds isolated from butter were strongly oxidase-positive. No oxidase-positive organisms were found in aseptically drawn milk. Oxidase-positive organisms were found in great numbers in molasses, corn silage, dust, decaying plant tissue, soil and surface waters and butter. Directions for making the oxidase test are given. O.F.G.

489. The Effect of pH on Growth and Gas Production by Streptococci and Lactobacilli. J. G. DAVIS AND C. C. THIEL, The Nat'l Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 455–463. 1939.

The pH ranges of growth of streptococci and lactobacilli in dextrose yeast casein digest broth was studied. Nearly all the group I and II streptococci grew at pH 8.8, but only the enterococci grew at pH 4.8. All group III types (heterofermentative) grew at pH 4.4. Only a few lactobacilli grew at pH 8.8 but most were able to grow at pH 4.0. *S. cremoris* could be differentiated from *S. lactis* by failure to grow at pH 9.2. S.T.C.

490. Bacteriophage-organism Relationships in the Group of Lactic Streptococci. H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., *10:* 403–409. 1939.

Methods are described for the isolation and purification of bacteriophages

active against lactic streptococci. The relationships between nine apparently distinct phage races and eleven strains of lactic streptococci were studied. The phages were found to show a tendency toward strain specificity, although some of the races attacked up to four different strains. Resistant strains usually were found to arise on continued incubation of lysed cultures. Cross resistant tests, in those cases where two or more phages act on one strain of streptococci indicated that the relationships between the phages does not follow any simple rule. S.T.C.

491. Biennial Reviews of the Progress of Dairy Science. Section B. Bacteriology and Mycology Applied to Dairying. J. Dairy Res., *10:* 515–549. 1939.

A review of literature published principally during 1937–1938 under the following headings:

I. Milk control
   Technique
II. Micro-organisms in milk and milk products
   (a) *Coli-aerogenes* group
   (b) Spore-bearing bacteria
III. Lactic acid and allied fermentations
   (a) Bacterial metabolism
   (b) Lactic acid bacteria
   (c) Starters
   (d) Cheese
   (e) Butter
IV. Pasteurization and other processes
   (a) Pasteurization
   (b) Other processes
   (c) Canned and dried milk products
   (d) Ice cream S.T.C.

492. Examples of Variation within Pure Cultures of Streptococcus cremoris. G. G. E. HUNTER, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., *10:* 464–470. 1939.

Variability within pure strains of *Streptococcus cremoris* was demonstrated. In one strain changes in acid producing power were made evident by changes in colony form. One variant failed to ferment lactose but was still susceptible to the specific race of bacteriophage and exhibited the same morphology as the parent culture. Variation was well marked within some strains, especially in regard to acid production, response to high temperature conditions, power to produce ropiness in milk and degree of resistance to phage attack. S.T.C.

493. The Enrichment of aerogenes-cloacae Types in Milk Held at Low Temperatures: with Observations on the Relative Rates of Growth of aerogenes-cloacae and B. coli Types in Milk at Different Temperatures. JAMES F. MALCOLM, Dept. of Bacteriology, The West of Scotland Agricultural College, Glasgow, Scotland. J. Dairy Res., *10:* 410–425. 1939.

Cultures of *B. aerogenes, B. oxytocus* and *B. cloacae* types were found as a rule to multiply much more rapidly when grown at 17° C. in sterilized, partly sterilized or raw milk than those of the *B. coli* types. Counts were made by the plate method using milk agar. With mixed cultures grown in milk the *aerogenes-cloacae* type multiplied more rapidly at 17 and 22° C. than the *B. coli*, while at 30 and 37° C. the reverse was the case. Twelve specimens of bovine feces were inoculated into raw milk and the cultures kept at 17° C. for 36 hours. *Aerogenes-cloacae* types occurring in the feces became enriched in the milk, the coliform flora at the end of the incubation period frequently consisting chiefly of these types.

The author explains the greater incidence of *aerogenes-cloacae* types in summer milk in Scotland as compared with winter milk, as due to enrichment of these types at the temperatures of holding commonly employed in the summer. Such enrichment does not occur in the winter since the holding temperature as a rule is so low that there is little or no proliferation of any coliform types. S.T.C.

BUTTER

494. A Discussion of Some Phases of Butter Deterioration. E. G. HOOD, Elgin Annex, Ottawa, Ont. Can. Dairy and Ice Cream J., *19:* 4, 54. 1940.

Real quality in butter means quality at the time the butter is consumed. Without quality cream there can be no quality butter. Flavors that appear in the cream are nearly certain to reappear in the butter unless specially treated and this treatment adds to the cost of manufacture. The age of the cream is one of the major factors controlling the quality of butter since time is required for harmful bacterial action and oxidation of the fat to take place. Not all sweet cream makes good flavored butter. Chemical deterioration resulting in off-flavor is due to oxidation, hydrolysis or catalysis of any of the constituents normally present in milk. Considerable quantities of butter now find their way into the second grade class as the result of surface flavor defects. Bacterial deterioration of the fat and protein of butter results in further flavor defects. O.F.G.

495. The Neutralization of Cream for Buttermaking. I. The Accuracy of Acid Reduction by Various Neutralizers. R. C. TOWNLEY AND I. A. GOULD. Can. Dairy and Ice Cream J., *19:* 5, 54. 1940.

The accuracy of acid reduction is dependent upon (a) the type of neu-

tralizer used, (b) the degree or extent of acid reduction, and (c) the temperature of pasteurization. Each of the neutralizers showed a distinct variation from the linear reduction of acidity. As the acidity ranges were lowered all of the neutralizers decreased in efficiency. Caustic soda and the limes were more efficient than the carbonates in reducing acidity throughout the entire range. The pH of the butter was always above that of the cream from which it was made. O.F.G.

496. "Vacreating" Cream. R. W. BROWN, Univ. of Manitoba, Winnipeg, Man. Can. Dairy and Ice Cream J., *19:* 3, 21. 1940.

Cream for buttermaking was treated in a "vacreator" which is essentially a flash pasteurizer working under reduced pressure. It is claimed that the cream reaches the pasteurizing temperature (201° F.) in one second and leaves the pasteurizing chamber in two seconds after entering. The cream passes from the pasteurizer to a second chamber under a vacuum of about 20 inches where volatile substances are drawn off. Butter made from "vacreated" cream of clean flavor and acidity was 1.08 points better in flavor score at the end of 4–6 weeks held at 45°–50° F. than was butter made from the same cream but pasteurized in the usual way. Butter made from high-acid stale cream and held under the above conditions showed an advantage of 2.08 points in flavor score in favor of the vacreated cream. The advantage for vacreated butter made from feed and weed flavored cream was 3.59 points and from metallic flavored cream, 2.46 points. More efficient neutralization was brought about in vacreated cream than in pasteurized cream. O.F.G.

497. Preparing Prize-winning Butter. L. C. THOMSEN, Univ. of Wis., Madison. Nat. Butter and Cheese J., *31:* 7, 10. July, 1940.

Suggestions are given to the man who is making butter for a contest. They cover the selection, standardization, pasteurization, cooling, ripening, and churning of cream; washing, salting and working the butter; finishing, filling, identification and shipment of the tub. W.V.P.

CHEESE

498. Factors Influencing Cheesemaking Methods and Yields. W. S. ARBUCKLE. Can. Dairy and Ice Cream J., *19:* 4, 60. 1940.

The author describes some of the physico-chemical phenomena and relationships in milk and in cheese. The roles of fat, casein, ash and lactose and their influence on color, flavor and body of cheese are discussed. Water has a definite influence on the body of cheese because it supplies a medium for the chemical breakdown of the casein during ripening. The problems of pasteurization of milk for cheesemaking, of mastitic milk, of the treatment of the cheese curd and of the influence of temperature on ripening are discussed. O.F.G.

499. The Bacterial Flora of New Zealand Cheddar Cheese. I.R. SHERWOOD, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., *10:* 426–448. 1939.

Seven hundred and twenty strains of lactic acid bacteria isolated from thirty-six typical cheddar cheeses were classified. *Streptobacterium plantarum* was found to be the dominant organism in New Zealand cheese. *Streptobacterium casei* occurs much less frequently, while betabacteria and betacocci are found in still smaller numbers. The flora of good quality cheese was found to consist mainly of one or two varieties of *Streptobacterium plantarum,* often associated with *Streptobacterium casei* or a small proportion of betabacteria.

Streptobacterium casei in general was found to be beneficial to cheese quality. The strains of *Streptobacterium plantarum* isolated were grouped in four varieties, mainly on the basis of sugar reactions. One variety was beneficial to cheese quality, another had very little effect, while the remaining two varieties produced serious defects—bad flavors, discoloration and, occasionally, open texture. The same defects were produced by the most of the strains of betabacteria and betacocci when present in large numbers.

S.T.C.

500. Lactic Acid Bacteria in Relation to Cheese Flavor. II. Observations on the Inoculation of the Milk Employed in Cheese Manufacture with Lactobacilli. I. R. SHERWOOD, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., *10:* 449–454. 1939.

Attempts were made to improve the flavor of cheese by inoculation of good quality pasteurized cheese milk with selected strains of *Streptobacterium casei* and *Streptobacterium plantarum.* Large inoculae imparted a sharpness to the flavor of the cheese especially during the early stages of ripening. The best results were obtained when the inoculum was such that the numbers of lactobacilli added were not much greater than the numbers of such organisms "naturally" present in raw milk, *e.g.,* 10 ml. of clotted culture to 80 gallons of cheese milk. The author suggests that under factory conditions the lactobacilli might be propagated by the incorporation in the "mother" starter (enriched with a vegetable extract) of suitable strains of *Streptobacterium plantarum.* S.T.C.

501. Mastitis and Cheese Milk. C. K. JOHNS, T. J. HICKS, AND C. A. GIBSON, Central Experimental Farm, Ottawa, Ont. Can. Dairy and Ice Cream J., *19:* 5, 19. 1940.

Yields of cheese from "abnormal" and "agalactive" milk were found to be lower than those from normal milk by about 5 per cent. No appreciable differences in the quality of the cheese from the 3 groups was noted. All

milks appeared perfectly normal and abnormalities were detected by biochemical or bacteriological tests. Udder injections increase the catalase content of milk and a method for measuring this enzyme seemed most promising for factory use. O.F.G.

502. Manufacture and Packaging of Cheese in a Labeled Consumer-size Package. H. L. WILSON, Bureau of Dairy Industry, U. S. Dept. of Agr. Milk Dealer, *29:* 10, 94–101. July, 1940.

A description is given of the proper methods to use in the processing, canning, and curing of cheese in order to insure a product of good uniform quality. The author concludes with the following statements: "High-acid cheese or cheese made from low-grade milk should not be canned. High-acid cheese never develops the characteristic flavor or improves with aging. The quality of the cheese varies directly with the quality of the milk from which it is made." C.J.B.

503. The Application of the Frozen Pack Method to Preservation of Soft Cheese. N. S. GOLDING AND MAX E. MORGAN, Dept. of Dairy Husbandry, Agr. Exp. Sta., State College of Washington, Pullman, Wash. Milk Dealer, *29:* 9, 42–46. June, 1940.

A report of experimental work to determine the application of the frozen pack method to the preservation of soft cheese. The authors conclude that Gervais cream cheese, Neufchatel and Neufchatel with pimento flavor are suited for frozen pack preservation. The limit of time for such storage under these conditions has not been determined, but a period of 10 weeks can be considered safe. Off-flavors and defective cheese, in general, will retain these defects during storage. C.J.B.

504. Fly Control in Cheese Factories. HUGH GLASGOW, N. Y. State Agr. Exp. Sta., Geneva. Nat. Butter and Cheese J., *31:* 7, 23. July, 1940.

Guarding the cheese factory from flies is made difficult by the number of species involved and their varied breeding habits. The house fly, blow flies, blue bottle flies and little house fly originate in filth or decaying animal matter. Immature midges live in ponds or streams while fruit flies or vinegar gnats develop in fermenting materials like fruits or milk refuse. The cheese maker should keep the flies out of the factory by keeping it dark by drawn shades and use of orange colored lights; by excluding them with screens supplemented by electrical screens; by eliminating breeding places; by using strong fly sprays and repellants around the outside whey tank and receiving platform; and by using odorless fly sprays and fly paper within the plant. W.V.P.

CHEMISTRY

505. Analysis of Commercial Fats and Oils. Report of Amer. Chem. Soc. Committee. Ind. and Eng. Chem., Anal. Ed., *12:* 7, 379–384. 1940.

This report gives details of methods of analysis which have been investigated and studied by the committee for a period of 2 to 3 years. The six methods which are recommended for adoption are: (1) the titer which gives the solidification point of fatty acids and in which a few changes have been made: (2) the modified Gardner break test which is applicable to crude soybean oil; (3) the detection of tristearin in lard which is applicable to the detection of foreign fats containing tristearin (beef fat) in unhydrogenated pork fats; (4) the Villavecchia test for the qualitative detection of sesame oil; (5) the method for calculation of the hydroxyl value for fat or wax which has been included with the acetyl value determination; (6) the smoke, flash and fire points applicable to animal and vegetable oils and fats.

B.H.W.

506. Assay of Vitamin A with the Photoelectric Colorimeter. R. B. French, Fla. Agr. Exp. Sta., Gainesville, Fla. Ind. Eng. Chem., Anal. Ed., *12:* 6, 351–352. 1940.

The accuracy of measuring vitamin A using the Cenco photelometer and the antimony trichloride reaction was investigated. The photelometer gave consistent, reproducible results in spite of the fact that the characteristic blue color faded rapidly. With close timing replicate determinations checked well.

B.H.W.

507. Making Casein Fiber. E. O. Whittier and S. P. Gould, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ind. Eng. Chem., *32:* 7, 906–907. 1940.

To make casein fiber casein is precipitated at a greater acidity than is ordinarily used in the manufacture of casein for other purposes. The kind and amount of acid used affects the fiber made from it. The casein is dissolved in an alkaline solvent such as NaOH, Na_3PO_4, NH_4OH. Salts of metals such as aluminum, calcium and barium should be present in the casein solution to increase the strength of the fiber. Fat acids such as oleic acid, linseed oil acids or others are added to the casein solution to increase flexibility. The casein solution is extruded through fine openings into a precipitating bath containing such an acid precipitant as sulfuric, phosphoric or acetic acid. The presence of 20 per cent glucose in the bath increases the speed of dehydration of the fiber. Formaldehyde or other aldehydes further increase the strength of the fiber and oil emulsions increase its softness and flexibility. Two examples of recipes giving relatively strong flexible fiber are given.

B.H.W.

508. Lactic Esters, Preparation and Properties. LEE T. SMITH AND H. V. CLABORN, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ind. Eng. Chem., *32:* 5, 692–694. 1940.

Since lactic acid may be manufactured from whey its utilization is important to dairy processors.

This paper reports the general methods of preparation and the properties of the 3 types of lactic esters which may be made from lactic acid. Some of the methods of preparation have commercial possibilities. A table giving some physical properties of 30 esters is given. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

509. Une Méthode Rapide pour l'Estimation des Properiétes Élastique et Plastique de la Caséine à la Préssure. (A Rapid Method of Estimation of the Elastic and Plastic Qualities of Rennet Casein.) G. GENIN. Le Lait, *20:* 291–296. May–June, 1940.

The use of casein in the plastic industries is hindered because of the marked variation which exists from one batch of raw casein to the next. C. A. Cooper (Brit. Plastics, March, 1939, p. 572) found that the Scott apparatus used by rubber manufacturers could be adapted to determine the deformations and elasticity of casein samples under heat and pressure.

Cylinders of casein are moulded under standard conditions and then subjected to three minutes pressure at 120° C. The temporary deformation, permanent deformation and elasticity can then be obtained by measuring the changes in height which occur in the test pieces.

Highly elastic caseins are difficult to mill and shape. There is no strict relationship between the elastic properties of casein and pH although most of the superior New Zealand and Australian samples gave higher values for both pH and ash content. O.R.I.

510. Le Controle Hygiénique du 'Lait Concentré Sucré.' (The Hygienic Control of Sweetened Condensed Milk.) C. A. CECILIA, Vet. School, Madrid. Le Lait, *20:* 271–279. May–June, 1940.

During the Spanish civil war, large quantities of sweetened condensed milk of foreign origin were used in Spain. Defects present in 149 samples studied by the author are reported upon. Cans were examined for swelling and leaking externally, and for odor, color, taste and abnormal viscosity after incubation. Fifty ml. of a 1:5 dilution was also examined by inoculation in peptone water, Breed count, plate count on glucose, agar, reductase, acidity and sediment.

In many cases, swollen cans contained rancid, fruity, cheesy or sour milk. Bacterial flora included cocci, Torula and spherical yeasts, spore-forming rods, and some moulds. Counts were much higher than are usually

reported for sweetened condensed milk. Excessive thickening usually accompanied high counts. Oily and tallowy flavors were only reported in five samples.

Incubation of suspicious cans for 48 hours and determination of acidity appeared to be the most reliable method of testing the quality of this product. O.R.I.

511. The Denaturation of the Soluble Proteins of Whey by Heat. O. R. IRVINE AND W. H. SPROULE, Ontario Agr. College, Guelph, Ont. Can. Dairy and Ice Cream J., *19:* 3, 62. 1940.

Pasteurization temperatures higher than 155° F. result in the denaturation of whey albumin. It was found that long holding times did not greatly increase the percentage of protein denatured except when the pasteurization temperature was 74.9° C. and the pH 6.35. An appreciable amount of protein denaturation may be expected even at a relatively low temperature of pasteurization. Denaturation can be greatly reduced if pasteurization of the whey can be carried out at a lower level of acidity. O.F.G.

512. Factors Affecting the Solubility of Milk Powders. IV. The Influence of Speed and Duration of Stirring on Solubility with Description of a Rapid Method for Solubility Determinations. J. R. HOWAT, J. A. B. SMITH, R. WAITE, AND N. C. WRIGHT, The Hannah Dairy Research Institute, Kirkhill, Ayr. J. Dairy Res., *10:* 498–514. 1939.

Increases in the speed and duration of stirring were found to increase the apparent solubility of milk powders. It was concluded, however, that 30 seconds shaking of the powder reconstituted in a 10 per cent mixture was sufficient to dissolve the truly soluble portion of the dried milk, but that the protein which has become denatured during the drying process tends to pass into solution with increased speed and duration of stirring.

The following method is suggested for solubility determinations: "1 g. of the powder to be tested is weighed into a 15 ml. centrifuge tube. About 2 ml. of water are added from a burette and the mixture stirred well with a glass rod which had been previously wetted. When all the powder has become thoroughly moistened, more water is added until a total of 9 ml. has been run in, the stirring rod being washed with the last few ml. of water. The tube is then stoppered and kept in a water bath either at 20 or 50° C. for 5 min. and is then shaken rapidly for 1 min. The shaking speed will affect to some extent the solubility figure finally obtained, but if the process be carried out as vigorously as possible, involving some 5–6 complete double excursions per second, very close agreement is obtained by different workers for the same sample. If it is desired to estimate the solubility at 50° C. the tube is shaken inside a container lined with cotton

wool to conserve the heat. The tube is then cooled to room temperature and centrifuged, the supernatant layer poured off as completely as possible (including the fat if the sample is a whole-milk powder), and its total solids content estimated by the rapid method of Golding. The ratio of the dissolved solids to the solids initially present (expressed as a percentage) is taken as an index of the solubility. The initial solids must be corrected for moisture content."

At higher solubilities the sediment volume found in the sediment method was shown to be a fairly reliable guide to small changes in solubility, but was much less accurate in the lower solubility ranges. S.T.C.

NOTE: This method is in variance with the accepted method recommended by the Dry Milk Institute.

DISEASE

513. Action of Gramicidin on Streptococci of Bovine Mastitis. R. B. LITTLE, R. J. DUBOIS, AND R. D. HOTCHKISS, Depts. of Animal and Plant Pathology and the Hospital, Rockefeller Inst. for Med. Res. Proc. Soc. Exp. Biol. and Med., *44:* 444. 1940.

An attempt was made to determine if gramicidin, an alcohol-soluble substance isolated from cultures of a sporulating bacillus, would destroy the streptococci causing mastitis when injected into the infected quarter. Repeated treatments of 2 animals suffering from chronic mastitis failed to eliminate permanently the streptococci from the infected quarters. Three cows with 9 infected quarters were treated with gramicidin. In 2 quarters repeated treatment failed to eliminate the streptococci while 5 treatments were required to sterilize one of the quarters. Repeated treatments resulted in a decreased milk flow. In the remaining 6 quarters (in 5 cases after a single injection) the streptococci disappeared without an appreciable decrease in milk secretion. It was concluded that "Before the effectiveness of gramicidin in the control of bovine mastitis can be determined, a larger number of animals must be treated and observed over a longer period of time." R.P.R.

FEEDS AND FEEDING

514. Chemical Changes in Phosphoric Acid Silage. EDOUARD PAGÉ AND L. A. MAYNARD, Cornell Univ., Ithaca, N. Y. Ind. Eng. Chem., *32:* 8, 1140–1143. 1940.

Grass silage, constituting of 62.2 per cent clovers, 19.8 per cent alfalfa, 16.2 per cent grasses and 1.8 per cent weeds and from 0 to 24 lbs. of 68 per cent phosphoric acid per ton of silage added to different layers, was subjected to chemical analysis after 8 to 9 months in the silo. The layers of silage were separated by waterproof rubber sheets and it was found that

the position of the layer markedly influenced the quality of the final product. To counteract this effect of position, for experimental purposes, the treatments applied to the lower layers were repeated in the upper half of the silo. The appearance and odor of all layers were good but analysis showed that the higher quality of some samples was due to the presence of lactic as well as phosphoric acid. The chemical evidence shows phosphoric acid to be of definite value as a preservative but that its action must be supplemented by a strong lactic acid production for best results. B.H.W.

515. The Effect of Increased Iodine Feeding upon the Iodine Content of Cow's Milk. N. L. MATTHEWS, G. M. CURTIS, AND J. H. MEYER, Dept. of Research Surgery, The Ohio State Univ., Columbus, Ohio. J. Dairy Res., *10:* 395–402. 1939.

The milk and blood iodine of a herd of thirty Guernsey and thirty Holstein cows was determined previous to and during prolonged increased iodine feeding to one-half of the herd. The increased amount of iodine was mixed with the grain ration, 3.2 mg. per cent of iodine as potassium iodide being added.

The blood iodine of the iodized cows was greatly and uniformly increased. The milk from the iodized cows contained 7 to 26 times as much iodine as that from the control cows. An average milk iodine of 80 mg. per cent was obtained from the iodized cows throughout a mid-year period of 5 months. During late spring, however, the milk iodine from both the iodized and control cows was unusually low. During early autumn the milk iodine from the iodized cows was low. S.T.C.

516. Grass Silage. G. BOHSTEDT, W. H. PETERSON, AND F. W. DAFFEE, Univ. of Wis., Madison, Wis. Circ. 299, 20 pages. May, 1940.

A popular treatise dealing with the characteristics of, methods of harvesting, preserving, cost of putting up and the feeding of grass silage. W.E.P.

517. Home-grown Grains and By-Products as Feeds for Dairy Cattle. D. L. FOURT AND F. W. ATKESON, Univ. of Idaho. Ext. Circ. 68. Revised April, 1940.

In addition to a general consideration of the characteristics of a good ration suitable grain mixtures are given for roughages of different protein contents. W.E.P.

518. Dehydrated and Sun-Cured Hay. S. I. BECHDEL, A. W. CLYDE, C. O. CROMER, AND P. S. WILLIAMS. Pa. Agr. Exp. Sta. Bull. 396. June, 1940.

Tests with two types of artificial driers are reported: the rotary drum,

high temperature style; and the conveyor, low temperature style. Hay of superior feeding value for dairy animals can be produced regardless of the weather. The cost of drying is as yet too high to compete with sun-cured hay for dairy stock, though it is being used considerably in poultry feeds. The crushing process for hastening natural drying has much promise of usefulness in the humid sections of the country since it reduces damage to hay by dews and rains. Under favorable conditions, hay cut with a crusher-mower in the morning can be stored that afternoon, whereas mower-cut hay must be left in the field until the next day. Author's Abstract

519. Bone Meal versus No Bone Meal in the Dairy Ration. S. I. Bechdel, P. S. Williams, J. F. Shigley, and A. A. Borland. Pa. Agr. Exp. Sta. Bull. 395. June, 1940.

A total of 117 lactation periods of 33 dairy cows was studied in a comparison of bone meal versus no bone meal in the concentrates fed. The data obtained on the weight and health of calves and the number of services necessary for conception are slightly favorable to bone meal. The results on milk production lead to the conclusion that the ordinary Pennsylvania dairyman, unless he has unusually high producers, does not need to add bone meal to the dairy ration. Author's Abstract

FOOD VALUE OF DAIRY PRODUCTS

520. Le Taux de la Vitamine C du Lait de Vache et son Importance dans l'Alimentation Infantile. (The Vitamin C Content of Cow's Milk and Its Importance in Infant Feeding.) M. Guigoz. Le Lait, *20:* 279–286. May–June, 1940.

Certain clinicians claim that children can be protected from scurvey with an intake of as little as 5 mgm. of ascorbic acid per day in contrast to a theoretical requirement of 20–25 mgm. suggested by other authorities. Since mother's milk is almost twice as rich in this vitamin as is the average cow's milk, it is suggested that the period of breast feeding builds up a reserve in the infant sufficient to protect it through the first few months of life. In such cases, cow's milk contains sufficient ascorbic acid to assure protection of the healthy infant against scurvy throughout the time that it is exclusively milk fed. A good bibliography of recent literature is included in this report. O.R.I.

521. The Effect of Light on the Vitamin C of Milk in Different Containers. J. Houston, S. K. Kon, and S. Y. Thompson, National Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 471–474. 1939.

Twice as much vitamin C survived in milk in wax impregnated cartons

exposed to the action of light as in clear glass bottles. Brown glass bottles showed little destruction, green glass bottles considerably more, although less than wax impregnated cartons. S.T.C.

522. Effect of Irradiated Milk on Storage of Nitrogen and Acid Base Minerals in Children. FRANCES C. HUMMEL, HELEN A. HUNSCHER, AND ICIE G. MACY, Children's Fund of Michigan, Detroit, Mich. Am. J. Diseases of Children, *58:* 753. 1939.

Observations were made on the mineral storage of eight children 5 to 8 years of age during test periods of 20 to 60 days, each child receiving first non-irradiated milk and later irradiated milk of the same kind and quantity. Three of the children were given fluid milk, 3 were given evaporated milk, and 2 were given a combination of both kinds.

From the standpoint of the average actual daily retention alone, it did not appear that the additional vitamin D in irradiated milk (90 to 96 and 180 to 190 U.S.P. units daily) had any nutritive advantage in the deposition of minerals. On the other hand, the children showed a more rapid rate of growth in length during the periods when vitamin D was the only factor added. The increase in rate of skeletal growth was, in general, accompanied by a higher calcium-phosphorus ratio in the retention. This indicates a stimulus to formation of bone and by the decrease in retention of nitrogen, phosphorus, and sulfur a shift from the formation of soft tissue to that of skeletal structure. From these cumulative results it seems justifiable to conclude that vitamin D supplements in the form of irradiated milk included in the dietary known to be nutritionally good and given to children who have good health not only exert a regulating influence on the growth impulses but stimulate skeletal development. W.H.R.

523. Influence of Fluid and Evaporated Milk on Mineral and Nitrogen Metabolism of Growing Children. HELEN J. SOUDERS, HELEN A. HUNSCHER, FRANCES C. HUMMEL, AND ICIE G. MACY, Children's Fund of Michigan, Detroit, Mich. Am. J. Diseases of Children, *58:* 529. 1939.

The nitrogen and acid-base mineral balance study was conducted on 3 healthy children 4½ to 6 years of age, receiving a basal diet of adequate requirements. During the first period of 25 to 40 consecutive days of metabolic study, 400 grams of plain fluid milk were ingested daily by each child. During the 20 to 25 days immediately following, a similar amount of dilute (1:1) evaporated milk was substituted and for the next 25 days irradiated evaporated milk was fed.

An added impetus to the formation of soft tissue resulted from the substitution of evaporated for fluid milk as shown by the parallel increases in retention of nitrogen, sulfur, and potassium.

When irradiated evaporated milk was included in the diet the increased calcium-phosphorus ratios of the retention, the higher levels and more consistent trends of the acid base balances, and the increase in rate of gain of recumbent length all indicated a more rapid and stable rate of formation of bone. W.H.R.

ICE CREAM

524. Using Frozen Fruit Pulps in Ice Cream Making. D. C. SORBER. Can. Dairy and Ice Cream J., *19:* 5, 22. 1940.

It is now possible by means of an inexpensive freezing process to preserve the important characteristics of fresh fruit for use in making ice cream at seasons of the year when the fresh fruit is not available. A long list of satisfactory varieties of fruit is given. Nothing but fully ripened soft fruit with high flavor should be used. Directions are given for the preparation of the fruit and the proportion of sugar to use. Contamination of the mix or of the fruit with copper should be avoided since several kinds of fruit intensify the reaction of copper in the development of oxidized flavor. Rapid handling is recommended to insure the preservation of the maximum quality that exists in fresh fruit. O.F.G.

525. Some Causes of Shrinkage in Package Ice Cream. ROLAND KOHLER, 2101 S. Los Angeles St., Los Angeles, Calif. Can. Dairy and Ice Cream J., *19:* 3, 26. 1940.

Factors which cause the shrinkage of ice cream are, (1) mix ingredients and mix composition, (2) processing mix, (3) freezing process, (4) hardening process, (5) transportation or delivery, and (6) storage and cabinets. Mix composition is the least expected and the hardest to control but it was found that the stability of milk solids-not-fat, especially those from condensed skim milk, play an important role in shrinkage. Tests indicated that adjustments of the stability of the proteins could be made through the addition of calcium, sodium, phosphates and citrates. Factors which tend to increase instability, and thus shrinkage, are high temperature pasteurization, homogenization at high pressures, the freezing process, especially high speed continuous freezing, and hardening at especially low temperatures. O.F.G.

526. A Discussion of Sweetening Agents for Ice Cream. P. H. TRACY, Univ. of Illinois, Urbana, Ill. Can. Dairy and Ice Cream J., *19:* 3, 58. 1940.

Sugar adds to the food value, improves palatability and lowers the freezing point of ice cream. The sweetening value of cane and beet sugar is the same. Dextrose, made from corn, has come to replace sucrose to some

extent in ice cream manufacture. It is more easily assimilated by the body but in water solution it has a sweetening value of about 70 as compared to 100 for sucrose. Its sweetening value is increased when used in conjunction with sucrose in ice cream. Its use will not seriously affect the time required to freeze and whip the mix but the drawing temperature will be about one degree lower. When used to replace about 25 per cent of the sucrose, it will have no detrimental effect on the flavor or body of ice cream. A new type of corn syrup, "Sweetose," has been developed recently that may have considerable use in the manufacture of ice cream. The body of ice cream containing "Sweetose" is noticeably smoother than the body of ice cream sweetened entirely with sucrose. The author recommends about 9 per cent honey with 8 per cent sucrose and no additional flavor to get the best honey flavor in ice cream. O.F.G.

MILK

527. Milk and the Public Health. R. O. Davidson. Can. Dairy and Ice Cream J., *19:* 4, 68. 1940.

The importance of safe milk for public consumption is emphasized. "Clean milk does not necessarily indicate a 'safe milk,' consequently something else, other than sanitary conditions, is required; that something is heat treatment of the milk to kill pathogenic organisms." The author feels that nutritive losses in the pasteurization of milk are not important. O.F.G

528. Safeguarding the Milk Supply of Large Cities. J. C. Geiger and B. G. Engle. Can. Dairy and Ice Cream J., *19:* 5, 30. 1940.

This is a summary of the needs for the methods of safeguarding the milk supply of large cities. The vital steps in offering the public a safe milk are:

(1) All milk and milk food products should be produced by healthy herds free from tuberculosis.

(2) All milk and milk food products should be produced from "Grade A" farms under the inspection service of the local department of health.

(3) All milk should be pasteurized with a rigid system of inspection of the plants. O.F.G.

529. Factors Affecting the Whipping Quality of Whipping Cream. C. J. Babcock, Bureau of Dairy Industry, Washington, D. C. Can. Dairy and Ice Cream J., *19:* 5, 40. 1940.

Raw cream is slightly superior for whipping purposes but the safeguards of pasteurization should be considered. Homogenization destroys the ability of cream to incorporate air and thus impairs its whipping quality. Aging will improve whipping quality but a period longer than 24 hours is not

recommended. Increasing the acidity does improve whipping quality. To get good whipping the temperature should not be above 50° F. O.F.G.

530. Titration des *B. Cole Aerogenes* dans les Laits. (Detection of Coli-aerogenes in Milks.) E. PIRAUX, Exp. Sta., Gembloux, Belgium. Le Lait, *20:* 257–271. May–June, 1940.

The presence of organisms of the *Escherichia-Aerobacter* group has significance in pasteurized milk, in butter, and in milk for cheese manufacture. Unless thermoduric strains are present, organisms of this type should not survive pasteurization. Positive differentiation between Escherichia and Aerobacter groups is difficult due to the many atypical strains found in nature.

A presumptive test for the presence of colon organisms must show the production of acid and gas in a liquid medium. Lactic acid production by lactics must be inhibited if growth of the colon types is to take place. Crystal violet, sodium formate, bile salts, etc., have been suggested as suitable antiseptics for this purpose.

In the study, tryptoflavine was found to be a satisfactory lactic inhibitor when used in a concentration of 1 or 2 parts per 100,000 parts of milk. One part of a 1 per cent neutral red solution in 100 parts of milk, was used as a reduction indicator. The milk was enriched by the addition of yeast extract at the rate of 2 per cent. In order to more accurately detect gas production the medium in the tubes was covered with a layer of melted paraffin. Incubation was at 37° C. for 24 to 48 hours.

If it is necessary to determine the presence of these organisms in low dilution, the sample can be diluted in physiological saline and the above medium prepared using sterile milk.

Confirmatory tests recommended include: Gram staining, or plating on Difco Violet Red Bile Agar, on Endo's medium or on Levine's medium. O.R.I.

531. La Réglementation du Lait Malpropre. (The Control of Unclean Milk.) L. HOTON. Le Lait, *20:* 287–291. May–June, 1940.

The advisability of attempting to legally define "clean" milk is discussed. Such a definition is very difficult and many primitive and unsatisfactory regulations are cited which have been retained in the statutes. Some of the more obselete statutes prohibit filtering. Several suggestions are put forward for revising these laws. O.R.I.

532. Milk and Child Welfare. A. B. SCHWARTZ, Chairman, Child Welfare Committee, State Medical Society of Wisconsin. Milk Dealer, *29:* 9, 110–113. June, 1940.

A brief history is given of the role which milk has played in child welfare. C.J.B.

533. What the Consumer Believes. EDWARD FISHER BROWN, Milk Research Council, New York City. Milk Dealer, *29:* 9, 114–117. June, 1940.

A psychological study of the attitudes of various lower-income group New York City housewives showed that: Most New York City housewives were favorably disposed or neutral toward their milk companies. More than two-thirds of the group, 719 out of 1,025, were generally satisfied with the milk companies' policies. Interviewers on this assignment encountered no positive hostility. A report is also given on the milk-buying habits, brand-buying habits, reason for brand selection, reactions to suggested innovations, familiarity with milk industry, and the comparative ranking of factors influential in milk buying. C.J.B.

534. Lipolysis in Raw Milk. Influence of Homogenization Temperature. I. A. GOULD, Mich. State College, East Lansing, Mich. Ind. Eng. Chem., *32:* 6, 876–877. 1940.

Raw milk which was quickly heated to 70°, 105°. 115°, 125°, 135°, and 145° F. and homogenized immediately at 1500 lbs. pressure showed lipolysis in each case. Maximum fat splitting occurred within the temperature range 105°–125° F. with only a slight lipolysis at 145° F. Samples of milk treated in the above manner but stored at 35°–40° F. for 72 hours underwent much greater lipolysis than samples not stored but the trend of results was the same. B.H.W.

535. The Cause and Control of Rancid Flavor in Milk. N. P. TARASSUK, Univ. of California, Davis, Calif. Can. Dairy and Ice Cream J., *19:* 3, 32. 1940.

Rancidity in milk is the result of the hydrolysis of milk fat by a lipolytic enzyme. This enzyme may be secreted into the milk by the cow or may come from bacteria growing in the milk. Lipase may be present in milk but may not be active. It may be activated by shaking or homogenization or by temperature manipulation. It was found possible to produce at will either a milk high in natural lipolytic activity or one free from it by merely changing from a dry feed to a green feed. Lipase action results in the lowering of surface tension and this characteristic may be used as a means of determining rancidity. Heating of the milk results in inactivation of the lipase. O.F.G.

536. Fluid Milk vs. Canned Milk. C. W. PIERCE, Dept. Agr. Economics, Pennsylvania State College, State College, Pa. Milk Dealer, *29:* 10, pp. 35, 63–64. July, 1940.

Statistics are given which show that the use of evaporated milk has increased rapidly in the United States. It is further shown that with the

increased use of evaporated milk there has been a gradual widening of the spread between the retail prices of fresh and evaporated milk. The author points out that a study of sales of milk from retail stores in New York City indicates that in low income areas and to a lesser extent in medium income areas there is a very definite relationship between the retail price differential and the sales of the two products.

The author states that: "The only logical conclusion is that an adjustment will have to be made in both the farm price and the distributor's margin if fresh milk is to compete effectively with evaporated milk."

C.J.B.

537. **Preservation of Devonshire or Clotted Cream.** N. S. GOLDING AND MAX E. MORGAN, Division of Dairy, Agr. Exp. Sta., State College of Washington, Pullman, Wash. Milk Dealer, *29:* 10, pp. 32, 67–69. July, 1940.

From a study of the application of the frozen pack method to the preservation of Devonshire cream the authors concluded that: "Since it is not a general practice to hold frozen foods more than six to eight months and our experiments show that Devonshire cream can be held for approximately these periods, it may be definitely concluded that clotted or Devonshire cream is quite suited to the frozen pack method of preservation."

C.J.B.

538. **Enzymes and Other Substances as Antioxidants in Milk.** D. H. NELSON AND C. D. DAHLE, Dairy Dept., Pennsylvania State College, State College, Pa. Milk Dealer, *29:* 10, 41–55. July, 1940.

Details are given of experimental work to determine the efficiency of enzymes and other substances as antioxidants in milk. This work is summarized as follows:

Certain substances were found to inhibit the development of the copper-induced oxidized flavor in milk. Results obtained with pure ascorbic acid agree with the reports of other investigators that it inhibits the development of the off-flavor, and the ascorbic acid itself practically disappears before an oxidized off-flavor appears. However, when fresh tomato juice or fresh orange juice is added to milk, the protection is greater than would be expected from the amount of ascorbic acid which they contribute. Oat flour was also found to inhibit the development of the copper-induced flavor even after the ascorbic acid, which it did not protect, had disappeared. Therefore, a copper-induced flavor can appear only when practically all of the ascorbic acid has disappeared but will not necessarily develop in the absence of ascorbic acid, especially when certain water-soluble antioxidants are present.

Citric acid failed to exhibit antioxidative properties when added to milk, although other investigators have found it effective in lard.

Addition of very small amounts of trypsin and steapsin preparations to milk was found to inhibit the development of copper-induced oxidized flavor. Since pepsin exhibited no antioxygenic properties, and since steapsin exhibited greater protective powers than did trypsin, it is believed that the beneficial effect of trypsin and steapsin is due to their action on the fatty material rather than to their action on the protein fraction of the milk or on the oxidation-reduction potential. Several attempts to destroy entirely the activity of steapsin by heat or by copper, were unsuccessful.

Gum guaiac exhibited antioxygenic properties in milk when used in much smaller concentrations than any of the antioxidants used. This antioxidant is very interesting because it is insoluble in water and only very slightly soluble in fat. This suggests that certain antioxidants may not need to diffuse into the substrate. Furthermore, since these small concentrations of gum guaiac do not inhibit the oxidation of ascorbic acid, they would not be expected to influence the oxidation-reduction potential.

Crude sugar was also found to inhibit the development of copper-induced flavor. C.J.B.

539. A Rapid Phosphatase Test. Adaption of Scharer's Modification to Pasteurizing Conditions in Great Britain; B. A Study of Factors Influencing the Reliability of the Test. R. ASCHAFFENBURG AND F. K. NEAVE, National Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 485–497. 1939.

A modification of Scharer's "10 minute field test" said to be applicable to British pasteurization conditions is described. The test requires less than 1½ hours for completion.

Experiments indicated a higher than normal phosphatase content in milk from cows suffering from mastitis both raw and after pasteurization. Unless more than 50 per cent of the cows contributing to a large bulk of milk are infected with mastitis it was found that this was unlikely to affect the results of the test. S.T.C.

540. Kay and Graham's Phosphatase Test. A. Modifications in Technique; B. Effect of Bacterial Growth. F. K. NEAVE, National Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *10:* 475–484. 1939.

It is suggested that the technique of the phosphatase test be modified as follows:

1. By the addition of sodium hexametaphosphate before placing the tubes in the boiling water bath. This makes it possible to eliminate the final filtration.

2. Avoid direct sunlight during that part of the test in which Folin's reagent is in use.

The pH of the milk samples tested was found to vary naturally before pasteurization from pH 6.5 to 6.9 without affecting the validity of the test.

The test was unaffected by the presence in milk of bacteria which produce reducing substances or which hydrolyze the substrate (sodium phenyl phosphate) unless the organisms are present in very large numbers.

S.T.C.

PHYSIOLOGY

541. H Ion Concentration of Various Fluids of the Genital Tract of the Cow. HENRY A. LARDY, W. D. POUNDEN, AND PAUL H. PHILLIPS, Dept. of Biochemistry and Veterinary Science, Univ. of Wisconsin. Proc. Soc. Exp. Biol. and Med., *44:* 517. 1940.

A study of the pH of the genital secretions of the cow showed the vagina to be slightly acid during diestrum (6.4) while it was slightly alkaline during estrum. The fluids of the cervix during estrum were slightly alkaline (8.3) while the fluids present in the uterus during estrum were slightly acid (6.8). R.P.R.

542. Effect of Volume Used for Injection in Micro-Assay of Prolactin. ROBERT W. BATES AND OSCAR RIDDLE, Carnegie Institution of Washington. Proc. Soc. Exp. Biol. and Med., *44:* 505. 1940.

Results were presented which indicate the importance of utilizing a constant volume of fluid when assaying the lactogenic hormone by the local crop-sac method. A minimum dose of prolactin for stimulation of the crop gland in 0.05 ml. has its effectiveness increased four fold when diluted to 0.50 ml. R.P.R.

MISCELLANEOUS

543. Influence of Government Activities upon the Dairy Industry. W. A. WENTWORTH, Dairy Industries Committee, Washington, D. C. Milk Dealer, *29:* 9, 118–125. June, 1940.

A discussion is presented of the effect of government activities, such as the Agricultural Adjustment Administration, Wagner act, the wage and hour law, and reciprocal trade agreements, on the dairy industry.

C.J.B.

544. Water Conditioning for Dairy Plants. G. A. RICHARDSON, Univ. of California. Can. Dairy and Ice Cream J., *19:* 4, 38. 1940.

The salts of calcium and magnesium are directly responsible for the majority of the troubles arising from the use of untreated water such as the formation of scale, sludge and milk stone. Softening water is the render-

ing of the calcium and magnesium unavailable for the formation of insoluble soaps or precipitates. This may be accomplished by (1) distillation, (2) heating raw water followed by settling and filtration, (3) lime treatment, (4) treatment with lime and soda, (5) base exchange, and (6) addition of alkalies such as caustic soda, soda ash, trisodium phosphate, sodium metasilicate, tetrasodium pyrophosphate, sodium bexametaphosphate and sodium tetraphosphate. Boiler water may be softened either by treatment with lime-soda or by base exchange. Water for condensers should be treated so as to keep the calcium salts in solution rather than to precipitate them. Water for washing operations should be softened by base exchange or treatment with some of the phosphates mentioned above. These phosphates act by the prevention of precipitates. O.F.G.

545. Water—Taste—Odor—Color. A. V. MOORE. Can. Dairy and Ice Cream J., *19:* 4, 52. 1940.

One of the prime factors involved in the establishment of a dairy enterprise is having an adequate supply of clean water. A water-lubricated pump is the most satisfactory. A potable water is not necessarily satisfactory for dairy use. Damaging flavor defects of butter are often traced to the water used in washing. Unsatisfactory sediment tests of certain dairy products may be traced to the water used in processing. Milk stone may not be directly due to water but it is aggravated by certain types of water. Cream feathering may be due to the mineral content of water. O.F.G.

546. Two-Metal Contact Corrosion. Its Causes and Prevention. H. E. TEPEL, Chief Engineer, Adalet Mfg. Co., Cleveland, Ohio. Milk Dealer, *29:* 10, pp. 36, 72–73. July, 1940.

A discussion is given of the effect of galvanic action (electrolysis) on the corrosion of metals. Where piping systems transmitting electrolytes such as tap water, brines, and other solutions are made up of dissimilar metals, it is necessary to use insulated couplings in order to prevent the rapid disintegration of one of the metals. C.J.B.

547. Cutting Production Costs through Planned Work Schedules. HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. Milk Dealer, *29:* 9, pp. 32–33, 59. June, 1940.

The author presents a "working and relief schedule" for medium-sized plants. This schedule is made up weekly and shows the work of each man for the week, his time off, who is to relieve him while off or in case of accident. It also shows the number of hours worked in the various departments of the plant. C.J.B.

548. Good Water—a Necessity in the Dairy Products Plant. K. G. WECKEL, Univ. of Wis., Madison. Nat. Butter and Cheese J., *31:* 7, 14. July, 1940.

All water in a dairy plant should preferably be of "drinking water" quality although re-used, lake, pond or river water may be used for economical reasons. All water used for rinsing equipment, washing of cheese curd or butter, standardization of condensed milk products, or in the making of fruit ices or fruit drinks should be free of undesirable organisms. The presence of foreign materials such as iron, copper or brass from equipment or metal "chore boys," sludge, grit and calcareous sedimentary accumulations, rubber or particles from disintegrating hose, oil contamination from water-pumping equipment, all may cause defects in dairy products or difficulties in cleaning operations. The mineral and organic matter of water varies with the season of the year, geologic source and availability of the water. A table shows the maximum range in concentration of constituents observed in the water supplies of 100 American cities. The metallic constituents may cause off-flavors in dairy products while "hardness" may affect undesirably the protein stability during sterilization of evaporated milk, feathering of cream in coffee, quality of casein used in glues, paints, plastics and paper sizing and may increase deposition of milk stone. Hard water tends to increase labor costs, consumption of soap and alkali, cleaning problems and problems of boiler management. Plenty of water available makes labor more efficient and aids in quality control. Dairy plant operators should appraise their water supplies in terms of costs of obtaining them, costs of using them, their bacteriological, chemical and physical characteristics and their influence on products and plant efficiency. Well and pump engineers, water engineers and chemists should be consulted for advice on efficiency of pump installations and purity of water supplies. W.V.P.

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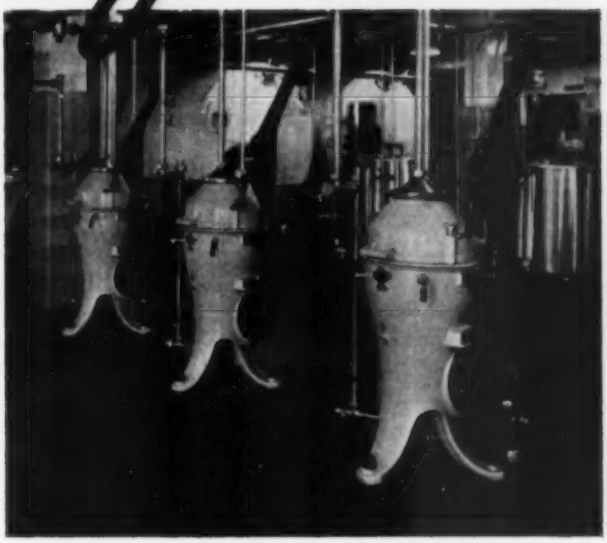

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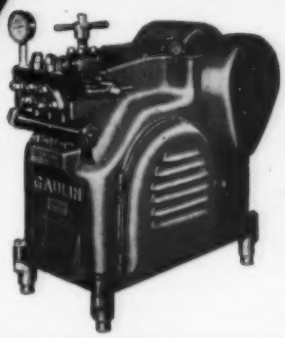

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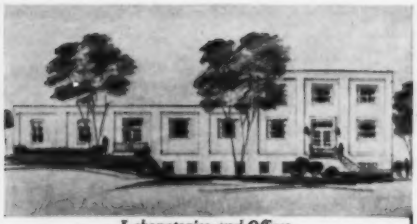

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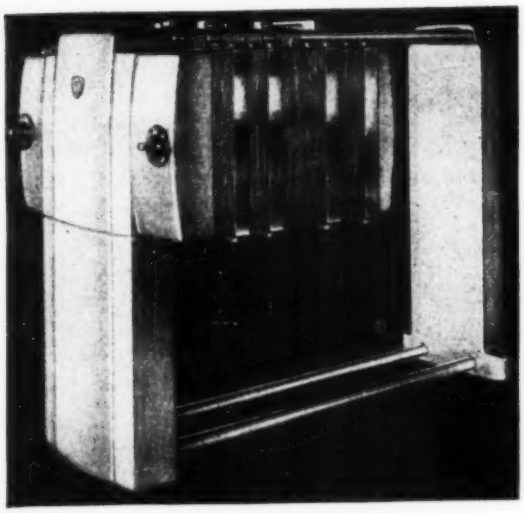

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